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EDITORIAL

A YEAR HAS PASSED

JUST over a year has passed since the war in Europe ended in victory for the Allies. As on previous occasions in our history, the successful termination of hostilities was the occasion for much relief and joy, and, naturally, hopes ran high for the future, a future in which there was to be freedom from want and freedom from fear. Have these two things been implemented to any degree? As regards freedom from fear there has been a welcome respite, especially in those lands most ravaged by war, but the spectacle of want stalks majestically throughout the whole world. There are wide areas where human beings, and, sad to relate, often those who strove most tenaciously for the conquest over evil things, lie in imminent danger not only of want, but of starvation, and, indeed, decimation.

During the war agriculture was resuscitated to become a number one priority. Great calls were made on the industry, and apparently wonderful results, often far beyond the most sanguine hopes, were achieved.

How is it, then, that the era of peace should find the world in such distress for the want of the products of an industry which had played such a noble rôle in war and achieved an economic standard higher than had ever been reached before?

The answer is a simple one. The volume of production had not kept pace with the rise in values. It was the latter which brought economic success to the

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producer. In this country agricultural production was increased by approximately 30 per cent., whereas values of the produce increased by approximately 104 per cent. Owing to the intensive ploughing up campaign, crop returns increased, but they increased at the expense of the livestock, and the balance between arable land and livestock suffered an upset. There has been a great decline in the sheep, pig and poultry population. The average milk yield has dropped. The consumption of raw milk has increased, which is good, but, on the other hand, the consumer has lost much of the milk which he obtained in manufactured products.

Now there is such a world shortage of grain for human needs that less and less can be spared for animal feeding, and before next harvest we may see a further decline in the numbers of livestock. All this makes a sad story, and one shudders when one thinks of what would have been the state of affairs if the war had continued into 1946 with its high demands, waste, and losses.

In meeting the new situation veterinary surgeons have a great part to play, even greater than their rôle in war. The animal population must be kept at maximum numbers and in good health. To do this our practitioners must collaborate with the farmers, not only in the prevention and treatment of disease, but in the wider sphere of husbandry. Much can be done in an advisory capacity on the problems of nutrition, so that optimum results can be obtained from restricted supplies. Breeding, hygiene and such things as the ecological balance of the stock on a farm are factors of major importance.

The practitioner should keep in close touch with the research workers, so that the proved useful findings of the laboratory may be implemented in the field at the earliest possible moment. The present is an opportune time to advise on culling. Animals below certain standards are non-economical; they are encumbrances, they eat food which could be more profitably used. Animals below certain minimal standards should be weeded out. The herd or flock may diminish in numbers, but quality will be enhanced.

Finally, as our difficulties are not confined to any one country, veterinary surgeons of all races should get together for the common good.

General Articles

SOME OBSERVATIONS ON THE BLOOD LEUCOCYTE PICTURE IN BOVINES DURING REACTION TO THE INTRADERMAL TUBERCULIN TEST

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Introduction

It has been recognised for some years in the United States of America that the routine tuberculin test employed there does not disclose all cases of tuberculosis in cattle. Hendershott and Johnston (1942) have reviewed the literature on blood leucocyte changes in tuberculosis and during reaction to the tuberculin test. They quote Bredeck (1929), who investigated the differential leucocyte counts in human beings before, and 24 hours after, the application of the subcutaneous tuberculin test. Bredeck found in tuberculous patients a marked "shift to the left" of the neutrophiles and a lowered lymphocyte count with, in some individuals, an extremely high monocyte count. Thiele (1938) studied the blood leucocyte picture in reacting and non-reacting cattle during the intradermal tuberculin test. He found an increase in the neutrophiles in reacting cattle 12 hours after injection, which reached its maximum within 32 hours; whereas in the blood of animals which showed increases in skin measurements not exceeding 3 millimetres there were no significant changes. Thiele suggested that a differential leucocyte count might be employed to assist in the diagnosis of tuberculosis by the intradermal test. Stasney and Feldman (1938) infected calves by the injection of virulent tubercle bacilli, and 8 weeks afterwards subjected them to a caudal fold tuberculin test. Within 24 to 72 hours following the injection of tuberculin there was a marked increase in the total leucocyte count, and this was accounted for mainly by an increase in neutrophiles.

Hendershott & Johnston then described their technique, in which they followed the lead of Bredeck. In the first instance the blood of non-reactors was examined before, and 24 hours after, the subcutaneous injection of 2 cubic centimetres of tuberculin, the later that of reactors. Although they did not always note the "shift to the left," they did observe a marked alteration in the neutrophilic-lymphocytic ratio, and stated that "in practically all cases in which lesions of tuberculosis were found on *post-mortem* examination the blood survey revealed a marked increase in the number of neutrophiles and a corresponding decrease in the number of lymphocytes." Results are shown of the application of the blood survey-tuberculin test to a total of 695 cattle in 12 herds suspected to have tuberculosis. The method revealed many more reactors than the intradermal tuberculin test used alone. Thus, of 86 blood-positive tuberculin test-negative cattle, 65 were slaughtered, and 56 revealed gross lesions of tuberculosis. Nine failed to show lesions, and 21 had not been slaughtered when Hendershott and Johnston recorded their results. In conclusion, these writers suggest that the technique described should prove of value in the detection of tuberculosis in cattle in advance of the time when the tuberculin test would disclose infection.

No mention is made by Hendershott and Johnston of an increase in the

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eosinophile count during reaction, although Holman (1944) states that eosinophilia is a feature of an allergic condition.

In this country, as compared with the United States, workers are more concerned with cattle which show so-called non-specific reactions to tuberculin, *viz.*, cattle allergic to mammalian tuberculin, but in which no trace of tuberculosis can be found at *post-mortem* examination. Such cases often arise in attested herds which have been free of tuberculin reacting cattle for considerable periods. Occasionally obvious " skin lesions " are present, and these are held to be responsible for sensitisation. More commonly, however, no explanation is forthcoming. A differential blood leucocyte count would appear to be rather impracticable as a field test, but in doubtful isolated cases it might usefully be employed in conjunction with the intradermal test if it were found to be more specific than the intradermal test alone.

The present writer decided to investigate the blood leucocyte changes during the double intradermal comparative tuberculin test, using Weybridge tuberculin, in both reacting and non-reacting cattle. An attempt was made in the case of one animal to determine when the maximum blood changes occurred, and to correlate intensity of skin reaction with the degree of leucocytic change in the blood.

Animals Used

(a) *Reactors.*

The animals used for this investigation were 10 pedigree Friesian heifers of ages ranging from 20 to 40 months. (Heifers were chosen for this experiment in preference to cows because, other things being equal, they would be less likely to be affected by intercurrent disease, for example, mastitis, which would alter the blood picture.) The heifers will be referred to as numbers 1 to 10. During the previous 5 months they had been housed with a diet of oat straw and roots, which was later supplemented by a mineral mixture and cod liver oil. The fæces of 1 to 7, inclusive, were examined for worm eggs on January 16, 1945, and moderate counts were found. Each animal was dosed with 30 g. of phenothiazine. Following this treatment, 1 heifer only, No. 5, showed any eggs in 2 g. fæcal samples. This would tend to exclude an eosinophilia which might result from endo-parasitic infestation. Heifers 1 and 10 were pregnant, 2 and 5 months respectively, at the time of the investigation, and the remainder had not been served. None showed clinical evidence of disease, but all were in poor condition, and one, No. 6, was undersized.

All the heifers had previously given positive reactions to the double intradermal comparative tuberculin test. Nos. 1 to 7 had reacted to the first test to be performed on them when they were 1 to 2 months old, and were believed to have become infected from the milk of a cow in the herd which was later found to have tuberculous mastitis. Since that time 3 more tests had been applied, and on each occasion pronounced reactions resulted from both avian and mammalian tuberculins, the latter being considerably in excess of the former. It was of interest to observe that the magnitude of both reactions, but particularly that of the avian, diminished with the passage of time.

Heifers 8, 9 and 10 were the oldest members of the group; they had not been treated with phenothiazine, and until February, 1945, had been negative to the tuberculin test. On this date, however, they gave pronounced reactions, with mammalian clearly in excess of avian.

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Thus it will be seen that in so far as sensitivity to tuberculin was concerned, the animals fell into 2 distinct groups, viz., (a) heifers 1 to 7, which had first reacted 18 months previous to the investigation, and in which allergy was on the decline, and (b) Nos. 8 to 10, which had first reacted 2 months previously, and in which it is reasonable to assume that sensitivity was ascending or had attained its maximum.

(b) *Non-reactors.*

These comprised 5 female animals in a "tuberculin tested" herd, and were of ages ranging from 4 months to 6 years. With the exception of one calf tested for the first time, all these animals had previously given no reactions to avian or mammalian tuberculins on several occasions. These animals will be referred to by their respective names. They were all in good condition.

Technique

Blood samples were taken by means of a hypodermic needle inserted into the raised jugular vein and caught in a dry watch-glass, a separate needle being used for each animal. Dilutions of 1 in 20 of 3 per cent. acetic acid were made, using a Piney pipette, and two smears of whole blood were at once made in the usual way. The first blood samples were taken at the time of the initial tuberculin injections, and subsequent ones at the twelfth, thirty-sixth and sixtieth hours. Skin fold measurements were recorded at each bleeding. The second tuberculin injections were made at the fifty-fourth hour. The tuberculins used were those prepared by the Ministry of Agriculture for use in the routine testing of animals under the Attested Herds Scheme, and were marked "Brew 12." 0.1 cubic centimetres of both avian and mammalian tuberculins were injected intradermally in the middle of the neck at two sites 5 inches apart.

Two total leucocyte counts of each diluted sample were made, using a Thoma cytometer, but where there was a discrepancy of more than 10 per cent. between the two counts a third was made and the mean taken. The results were expressed as the number of leucocytes per cubic millimetre of whole blood. Blood smears were stained by Leishman's method, and two separate hundreds of leucocytes differentiated. Again, in those cases in which appreciable discrepancy occurred a third count was made and the mean taken.

In the non-reactors the technique adopted was the same, except that the second injections of tuberculin were given at the forty-eighth hour and the final bleeding taken at the fifty-fourth hour. An improved cytometer of the Turck type was used, and the tuberculins were Ministry of Agriculture, marked "Brew 16."

Results Obtained

(a) *Reactors.*

Total and differential leucocyte counts, together with skin measurements, are shown in Tables 1, 2, 3 and 4.

(i) *The total leucocyte count.*

The average initial count was 6,580. Individual counts were all within the accepted normal range (8,000 plus/minus 2,000), except that of heifer No. 6, which was 4,000. (This animal gave a count of 3,900 on January 16, 1945, when phenothiazine was administered; she was undersized, but no cause to account for the leucopena or her failure properly to grow was discovered.)

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TABLE 1.

Blood picture and skin fold measurements at time of first injection of tuberculins.

Identification	Leucocytes	Neutrophiles Bands.	Neutrophiles Polys.	Lymphs.	Monocytes	Eosinophiles	Bas.	Skin measurements Avian	Skin measurements Mammalian
1	6,200	0.5	15.0	62.5	5.5	15.0	1.5	7 mm.	6 mm.
2	6,000	3.5	13.5	70.5	7.5	5.0	0.0	4 „	5 „
3	8,400	0.0	24.5	65.0	4.0	5.5	1.0	4 „	4 „
4	6,000	0.5	10.5	74.5	4.5	8.5	1.5	4 „	4 „
5	7,300	1.0	35.5	50.0	5.0	8.5	0.0	4 „	4 „
6	4,000	1.0	20.5	65.5	8.0	5.0	0.0	5 „	5 „
7	7,000	1.0	27.0	63.0	3.5	5.5	0.0	5 „	5 „
8	6,400	3.5	15.5	62.0	6.0	12.0	1.0	6 „	6 „
9	7,000	2.0	11.5	78.5	3.5	4.5	0.0	5 „	5 „
10	7,500	2.5	29.9	57.0	3.5	7.0	0.0	6 „	7 „
Average	6,580	1.5	20.3	64.8	5.1	7.8	0.5	5 „	5.1 „

TABLE 2.

Blood picture and skin fold measurements 12 hours after first injection of tuberculin.

Identification	Leucocytes	Neutrophiles Bands.	Neutrophiles Polys.	Lymphs.	Monocytes	Eosinophiles	Bas.	Skin measurements Avian	Skin measurements Mammalian
1	9,400	2.0	37.5	47.5	5.5	7.5	0.0	8 mm.	10 mm.
2	12,200	7.5	39.0	45.5	1.0	6.5	0.5	7 „	12 „
3	11,900	1.0	30.0	61.5	3.0	4.0	0.5	4 „	8 „
4	7,600	2.5	36.5	51.5	3.0	6.0	0.5	9 „	8 „
5	8,900	6.5	49.0	38.0	1.0	5.0	0.5	5 „	7 „
6	8,700	7.5	45.0	38.5	6.0	2.5	0.5	5 „	6 „
7	11,100	5.5	43.0	44.0	1.5	6.0	0.0	8 „	9 „
8	7,900	5.5	28.5	60.0	3.0	3.0	0.0	8 „	13 „
9	8,900	3.5	31.5	58.0	1.5	5.0	0.0	5 „	11 „
10	10,200	5.0	43.5	43.5	0.5	7.5	0.0	9 „	14 „
Average	9,680	4.7	38.3	48.8	2.6	5.3	0.3	6.8 „	9.8 „

TABLE 3.

Blood picture and skin fold measurements 36 hours after first injection of tuberculin.

Identification	Leucocytes	Neutrophiles Bands.	Neutrophiles Polys.	Lymphs.	Monocytes	Eosinophiles	Bas.	Skin measurements Avian	Skin measurements Mammalian
1	9,600	2.5	22.0	59.0	6.5	9.5	0.5	11 mm.	14 mm.
2	6,500	2.5	21.0	66.5	5.5	4.5	0.0	9 „	14 „
3	10,500	1.0	28.0	67.0	1.0	3.0	0.0	5 „	11 „
4	6,400	1.0	10.5	72.5	6.0	9.5	0.5	8 „	11 „
5	8,100	1.0	31.0	59.0	3.5	5.5	0.0	5 „	8 „
6	6,300	1.0	21.0	63.5	6.5	6.5	1.5	5 „	9 „
7	7,400	2.0	30.5	58.0	2.5	7.0	0.0	9 „	11 „
8	6,300	6.0	24.5	58.5	4.5	6.5	0.0	8 „	21 „
9	10,600	0.5	18.0	73.0	4.0	4.5	0.0	8 „	22 „
10	10,300	1.5	34.5	56.5	4.0	3.5	0.0	9 „	15 „
Average	8,200	1.9	24.1	63.3	4.4	6.0	0.3	7.7 „	13.6 „

TABLE 4.

Blood picture and skin fold measurements 60 hours after first injection and 6 hours after second injection of tuberculin.

Identification	Leucocytes	Neutrophiles Bands.	Neutrophiles Polys.	Lymphs.	Monocytes	Eosinophiles	Bas.	Skin measurements Avian	Skin measurements Mammalian
1	12,100	8.0	42.0	37.0	3.5	9.5	0.0	14 mm.	19 mm.
2	7,200	9.0	15.5	62.5	6.0	7.0	0.0	12 „	19 „
3	8,500	6.5	30.5	51.0	4.5	6.5	1.0	8 „	15 „
4	8,000	3.5	42.5	46.0	3.0	5.0	0.0	11 „	20 „
5	10,200	3.5	45.0	43.5	2.0	5.5	0.5	8 „	16 „
6	5,800	2.5	35.5	44.0	9.0	7.5	1.5	8 „	16 „
7	8,700	7.5	42.0	41.0	3.0	6.5	0.0	10 „	20 „
8	8,600	13.0	40.5	36.5	2.5	7.5	0.0	10 „	21 „
9	10,800	4.0	33.0	52.0	5.5	5.5	0.0	11 „	27 „
10	8,900	2.5	35.0	50.0	3.5	8.5	0.5	8 „	20 „
Average	8,880	6.0	36.2	46.3	4.2	6.9	0.4	10.0 „	19.3 „

At 12 hours after the initial tuberculin injections the average total count was 9,680. This figure also is within the normal range, but is an increase over the initial average of nearly 50 per cent. Individually, all the heifers showed considerable increases, and in the cases of 2 and 6 these increases were over 100 per cent.

At 36 hours, the average, although still elevated (8,200), was declining towards the pre-injection level. Heifers 1, 9 and 10, however, gave counts higher than at the twelfth hour. (Nos. 9 and 10 were both in the recently infected group.)

At 60 hours, that is, 6 hours after the second injections, the average count was 8,880, a figure less than that at the twelfth hour, but greater than that at the thirty-sixth hour. When considered individually, 3 heifers—Nos. 3, 6 and 10—gave decreased counts as compared with the thirty-sixth hour.

(ii) *The differential count.*

Neutrophiles. The average initial count was 21.8 per cent. of the total, of which 1.5 per cent. were of the non-segmented type ("bands"), but 5 animals, Nos. 1, 2, 4, 8 and 9, gave figures well below the normal, No. 4 being extremely low, viz., 11 per cent. Heifers Nos. 2 and 8 each showed 3.5 per cent. of "band" neutrophiles. At the twelfth hour the average was 43 per cent., of which 4.7 per cent. were "bands." This represents a 100 per cent. increase in neutrophiles, with a slight "shift to the left." Individually, 2, 5, 6, 7 and 10 showed marked neutrophilia with appreciable "shifts to the left." Heifer No. 4 showed a 200 per cent. increase in neutrophiles.

By the thirty-sixth hour the neutrophile percentage had fallen to 26.0, and the number of "bands" had also fallen to 1.9 per cent. Individually, no animal showed notable neutrophilia, although No. 8 showed 6 per cent. of "bands."

At the sixtieth hour the average percentage had risen again to 42.2, of which 6 per cent. were "bands," a figure which closely approximates that obtaining at the twelfth hour, but with a slightly more marked "shift to the left." If the results are examined individually at the sixtieth hour it will be seen that 5

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animals, viz., Nos. 1, 4, 5, 7 and 8, showed definite neutrophilia, and that the counts were increased from 100 to 300 per cent. over the initial levels, and that heifer No. 8 showed 13 per cent. of "bands."

Lymphocytes. The average initial count was 64.8 per cent. of the leucocytes. Individually, 3 animals, Nos. 2, 4 and 9, showed lymphocyte percentages above the upper limit of the normal range—52 plus/minus 15—namely, 70.5, 74.5, 78.5 respectively. At 12 hours after injection of tuberculin the average lymphocyte percentage had fallen to 48.8, a figure which is still within normal range, but which represents a fall of approximately 25 per cent. as compared with the initial level. At 36 hours the lymphocyte average had risen again almost to the initial level—63.3 per cent. At 60 hours the figure had dropped again to just below that for the twelfth hour—46.3 per cent.

Monocytes. The monocyte averages were at all times within the accepted normal range, and were 5.1, 2.6, 4.4 and 4.2 per cent. at the respective bleedings.

Eosinophiles. The initial eosinophile average was 6 per cent. of the leucocytes. Individually, all the animals showed eosinophile percentages which fell within the accepted normal range of 10 plus/minus 7 per cent. At 12 hours there was little change—5.3 per cent. Animal No. 6 showed 2.5 per cent. only at this stage. At 36 hours the eosinophile average was again 6 per cent., and at 60 hours the figure was 6.9 per cent. At no time during the investigation did any individual show eosinophilia.

(iii) *Skin reactions.*

All 10 animals gave clear-cut reactions to mammalian tuberculin, and this was manifest in the majority at the twelfth hour and increased progressively at the thirty-sixth and sixtieth hours. In all cases the skin measurement at the sixtieth hour was three or more times as great as at the initial reading, and the reactions were quite diffuse. Each animal reacted also to avian tuberculin, but not to the same extent as to mammalian.

(b) *Non-reactors.*

Total and differential leucocyte counts, together with skin measurements, are shown in tables 5, 6, 7 and 8.

(i) *The total leucocyte count.*

The average initial count and those at the twelfth, thirty-sixth and fifty-fourth hours were 8,860, 8,276, 8,020 and 8,140 leucocytes per cubic millimetre respectively. It will be seen that no significant changes occurred.

(ii) *The differential count.*

Neutrophiles. The average counts were 24.6, 27.2, 22.9 and 26.6 per cent., including 1.6, 2.2, 1.8 and 1.9 per cent. "bands."

Lymphocytes. Lymphocytes averaged 66.7, 63.7, 65.9 and 65.3 per cent.

Monocytes. Monocytes averaged 2.3, 3.7, 4.2 and 3.6 per cent.

Eosinophiles. Eosinophiles averaged 6.1, 5.3, 6.8 and 4.2 per cent.

The slight changes noted in the differential counts are also considered insignificant.

(iii) *Skin reactions.*

No animal showed an increase in skin measurement at the avian or mammalian site greater than 2 millimetres at any time during the investigation.

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TABLE 5.

Blood picture and skin fold measurements at time of first injection of tuberculin.

Identification	Leucocytes	Neutrophiles Bands.	Neutrophiles Polys.	Lymphs.	Monocytes	Eosinophiles	Bas.	Skin measurements Avian	Skin measurements Bovine
Floss	8,650	1.5	29.0	56.0	2.5	11.0	0.0	7 mm.	7 mm.
Bianca	10,850	2.5	22.0	73.5	1.5	0.0	0.5	4 „	3 „
Troubadour	7,550	0.5	25.5	61.5	3.0	8.5	1.5	5 „	5 „
Nephetiti	9,700	2.0	16.5	72.5	3.5	5.5	0.0	9 „	11 „
Walnut	7,550	1.5	22.0	70.0	1.0	5.5	0.0	8 „	8 „
Average	8,860	1.6	23.0	66.7	2.3	6.1	0.3	6.6 „	6.8 „

TABLE 6.

Blood picture and skin fold measurements 12 hours after first injection of tuberculin.

Identification	Leucocytes	Neutrophiles Bands.	Neutrophiles Polys.	Lymphs.	Monocytes	Eosinophiles	Bas.	Skin measurements Avian	Skin measurements Bovine
Floss	7,750	2.5	28.0	52.0	5.5	12.0	0.0	9 mm.	8 mm.
Bianca	10,350	1.5	14.5	80.0	3.0	1.0	0.0	4 „	3 „
Troubadour	6,330	4.0	24.5	63.5	2.0	5.5	0.5	5 „	5 „
Nephetiti	9,650	2.0	27.5	63.5	2.5	4.5	0.0	9 „	11 „
Walnut	7,300	1.0	30.5	59.5	5.5	3.5	0.0	8 „	8 „
Average	8,276	2.2	25.0	63.7	3.7	5.3	0.1	7.0 „	7.0 „

TABLE 7.

Blood picture and skin fold measurements 36 hours after first injection of tuberculin.

Identification	Leucocytes	Neutrophiles Bands.	Neutrophiles Polys.	Lymphs.	Monocytes	Eosinophiles	Bas.	Skin measurements Avian	Skin measurements Bovine
Floss	7,150	1.0	23.5	55.5	5.5	14.5	0.0	9 mm.	8 mm.
Bianca	10,300	0.5	12.5	85.5	1.5	0.0	0.0	4 „	4 „
Troubadour	6,900	2.5	24.5	60.5	5.5	6.0	1.0	5 „	5 „
Nephetiti	8,800	3.0	23.0	63.0	4.5	6.5	0.0	9 „	11 „
Walnut	6,950	2.0	22.0	65.0	4.0	7.0	0.0	8 „	9 „
Average	8,020	1.8	21.1	65.9	4.2	6.8	0.2	7.0 „	7.2 „

TABLE 8.

Blood picture and skin fold measurements 54 hours after first injection of tuberculin, and 6 hours after second injection.

Identification	Leucocytes	Neutrophiles Bands.	Neutrophiles Polys.	Lymphs.	Monocytes	Eosinophiles	Bas.	Skin measurements Avian	Skin measurements Bovine
Floss	7,450	0.5	33.5	54.5	2.5	9.0	0.0	8 mm.	8 mm.
Bianca	10,550	5.0	14.5	75.5	4.5	0.0	0.5	4 „	4 „
Troubadour	7,150	1.5	30.0	59.0	4.5	5.0	0.0	6 „	5 „
Nephetiti	8,200	2.5	25.0	63.5	3.5	4.5	1.0	10 „	11 „
Walnut	7,350	0.0	20.5	74.0	3.0	2.5	0.0	9 „	9 „
Average	8,140	1.9	24.7	65.3	3.6	4.2	0.3	7.4 „	7.4 „

It was now proposed to determine at what stage during the reaction to tuberculin the maximum change occurred in the neutrophilic-lymphocytic ratio, and to study the relationship between skin reaction and degree of leucocytic response.

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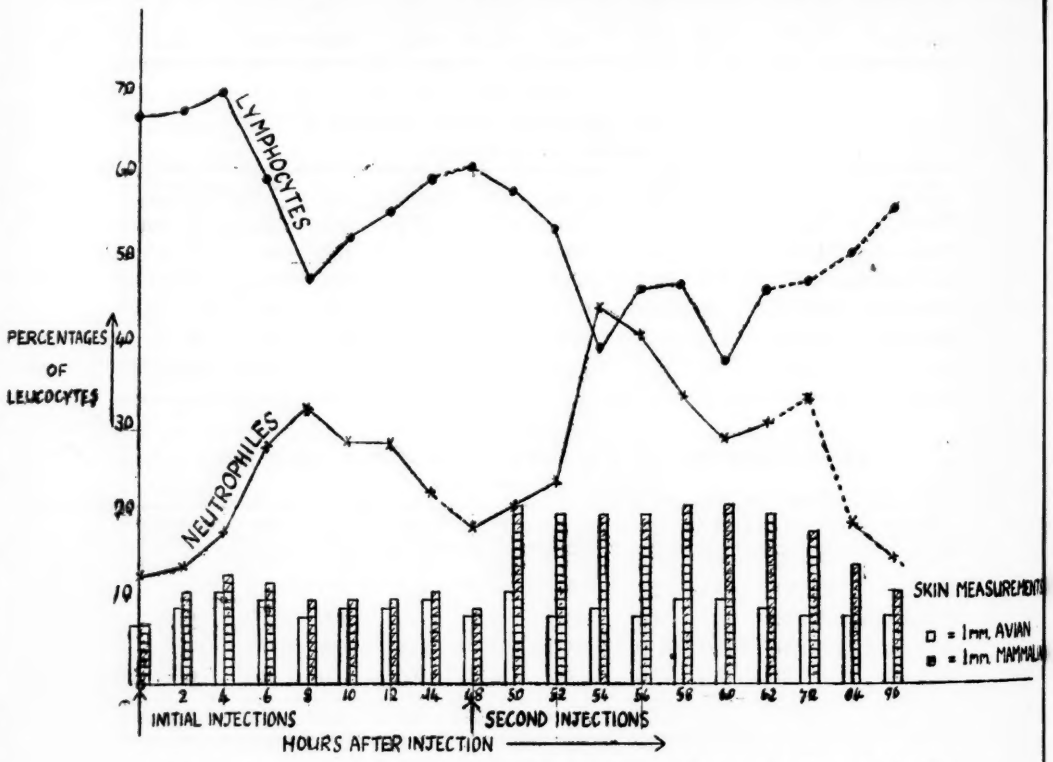
For this purpose animal No. 6 of the reacting group was used. This heifer was injected with avian and mammalian tuberculin (Ministry of Agriculture—"Brew 16") 7 months after the group investigation already described. By this time it had grown considerably and improved in condition. Blood smears and

LYMPHOCYTE AND NEUTROPHILE PERCENTAGES AND SKIN MEASUREMENTS DURING THE DOUBLE INTRADERMAL COMPARATIVE TUBERCULIN TEST.



total leucocyte counts were made and skin readings taken at the time of the first injections, and every 2 hours afterwards, until the maximum blood changes occurred. Similar observations were made at the second tuberculin injections and at 2 hourly intervals afterwards. The results are given in Table 9 and shown graphically in Diagram above.

It will be seen from the diagram that two peaks occurred in the skin readings, viz., at 4 hours after the initial injections and at 50 hours, i.e., 2 hours after the second injections. Similarly, the blood reaction was biphasic and maximal 8 hours after the initial injections and at 54 hours, i.e., 6 hours after the second injections. Thus in each case the maximum skin reading occurred 4 hours before the maximum blood change. It was also observed that the blood picture tended to return to normal more quickly than the skin.

It was interesting to note that at 60 hours the eosinophile count rose sharply to 29 per cent. of the leucocytes.

TABLE 9.

Detailed study of one animal (Heifer No. 6) during reaction to the double intradermal comparative tuberculin test. Observations made at 2-hour intervals, except where otherwise stated in table.

Total Leucocytes c/mm.	Neutrophiles % Bands.	Polys.	% Lympho-cytes	% Mono-cytes	% Eosino-philes	% Baso-philes	Skin measurements Avian	Mammalian
Initial.								
7,850	2.5	10.5	67.5	5.0	14.5	0.0	7	7
6,075	0.5	13.5	68.0	5.0	12.0	1.0	9	11 S.D.
7,450	1.5	16.5	70.5	1.0	10.0	0.5	11 D.	13 D.
8,600	3.5	24.5	60.0	3.0	9.0	0.0	10 D.	12 D.
8,325	4.0	28.5	48.0	3.5	15.0	1.0	8	10 D.
8,250	2.0	26.5	53.0	3.0	14.5	1.0	9	10 D.
7,625	1.0	27.5	56.0	3.5	10.5	1.5	10 D.	11 D.
7,600	0.5	22.0	60.0	3.0	12.0	2.5	9	10 D.
48 *hours.*								
6,325	1.0	17.5	61.0	3.5	16.0	1.0	8	9 S.D.
8,450	1.0	20.0	58.0	4.0	16.0	1.0	11 D.	21 D.P.
7,675	1.0	23.0	53.5	4.0	17.0	1.5	8	20 D.P.
9,975	5.0	39.5	39.5	1.5	13.5	1.0	9	20 D.P.
9,275	1.5	39.5	46.5	1.0	11.0	0.5	8	20 D.P.
13,000	0.5	33.5	47.0	4.0	13.5	1.5	10 D.	21 D.P.
9,650	3.0	26.0	38.0	2.0	29.0	2.0	10 D.	21 D.P.
8,525	3.0	27.5	46.5	3.5	19.0	0.5	9	20 D.P.
72 *hours.*								
6,425	2.0	32.0	47.5	2.5	14.5	1.5	8	18 D.
84 *hours.*								
7,400	1.0	18.0	61.0	4.5	15.0	0.5	8	14 D.
96 *hours.*								
7,650	0.5	14.5	66.5	3.5	14.5	1.5	8	11 S.D.

Discussion

Leucocytosis.

This investigation showed that the intradermal injection of tuberculin into reacting cattle was followed in 12 hours by a marked increase in the total leucocyte count. This increase tended to subside after 36 hours, but at 6 hours after the second injection of tuberculin it recurred.

Following intradermal tuberculin injections into non-reacting cattle, no significant changes occurred in the total counts.

Neutrophilia.

Coincident with the changes in the total leucocyte counts in the reacting group were marked alterations in the relative proportions of the various leucocyte types as shown by the differential count. On the average an increase in total leucocytes was accompanied by an increased percentage of neutrophiles and a decreased percentage of lymphocytes. That the myeloid tissues were stimulated specifically by tuberculin was shown by the appearance in appreciable numbers of immature neutrophiles in the circulating blood. These changes resulted in a significant alteration of the lymphocytic-neutrophilic ratio. Instead of a normal

ratio of approximately 2 to 1 (3:1 in the cases under consideration) the ratio was narrowed at 12 hours, and again at 60 hours, to approximately 1:1. This was considered to be the most important finding in this investigation, and is in harmony with the results obtained by Hendershott and Johnston.

In the 5 non-reacting cattle only insignificant changes in the neutrophile and lymphocyte percentages occurred.

Eosinophile count.

No eosinophilia followed the injection of tuberculin in the group investigation of the reacting cattle. This was the second important finding in the investigation. On the other hand, in the case of the individual study of animal No. 6, an eosinophile count of 29 per cent. was noted at the sixtieth hour. From this it would appear possible that eosinophilia might have been established in the group investigation if bleedings had been performed more often during the reaction to tuberculin.

Neutrophilia in relation to skin reaction.

Whilst increase in skin measurement coincided with neutrophilia at 12 hours and 60 hours, no constant correlation between intensity of skin reaction and degree of neutrophilia was observed. Also at the thirty-sixth hour, when the skin continued to increase in thickness, neutrophilia was subsiding. In the individual study of heifer No. 6 the maximum skin reading preceded the maximum blood change after the initial and second tuberculin injections by 4 hours, and the skin reaction was maintained longer than the blood change.

Neutrophilia in relation to ascending or descending allergy.

The more recently infected group of heifers, Nos. 8 to 10, showed similar leucocytic changes to the group 1 to 7, which were more distantly infected. Marked variations occurred in individual members of each group.

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Having established a definite association between blood leucocyte changes and capacity to react to the intradermal tuberculin test, it now remains to investigate whether the changes in the blood picture are simply another expression of tuberculin allergy or whether they are related to tuberculous infection. Work along these lines is proceeding.

Summary

(1) Total and differential leucocyte counts were made before, and at intervals during, the reaction to the double intradermal comparative tuberculin test in a group of 10 heifers, and in 5 non-reacting animals.

(2) The initial and second tuberculin injections were followed by marked increases in the total leucocyte counts and by a significant narrowing of the lymphocytic-neutrophilic ratio in the reacting group, but no such changes occurred in the non-reacting group.

(3) In one reacting animal investigated at 2 hourly intervals during the tuberculin test the maximum blood change occurred 6 hours after the second injection of tuberculin.

(4) Eosinophilia did not occur except in the one animal studied individually, where the count was 29 per cent. at 12 hours after the second tuberculin injection.

(5) Neutrophilia was accompanied by an increase in skin measurement, but no proportional relationship was found to exist between them. The greatest change in the blood picture occurred later than the maximum skin reaction.

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(6) The leucocytic changes in animals which had been reacting to the tuberculin test for 1½ years were similar to those which occurred in reactors of a few months' duration.

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THE TOXICITY OF SODIUM CHLORIDE FOR FOWLS

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Introduction

A SEARCH of the literature shows that comparatively little detailed work has been carried out on this subject. Salt poisoning is frequently suspected by poultry-farmers, particularly domestic poultry-keepers, as being the cause of any sudden mortality, although most of the available evidence shows that sodium chloride is not markedly toxic for poultry.

Where there is definite evidence that mortality has occurred in fowls following an excessive dose of salt in their ration, e.g., brine used for preserving vegetables or pickling pork, a diagnosis of salt poisoning seems justified. Cases of this type have been reported: Edwards (1918), Richardson (1942). During routine post-mortem diagnosis, cases are frequently encountered where mortality in adults and growing stock has occurred and in which the usual examinations have been concluded with negative results, although the accompanying history states that the owners suspect the birds to have been poisoned by excess of salt in the ration or drinking water; a case of this type occurred recently where the premises adjoined an aerodrome, on which brine had been used to disperse snow from the runways, and it was suggested that the melted snow and brine had drained into the ditches from which the birds drank; a similar case was that of a local domestic poultry-keeper, where 12 out of 13 hens died after eating a mash containing salted beans.

Suffran (1909) and Gallagher (1919), using individual birds, concluded that the lethal dose was 4 grams per kilogramme body-weight (2 drachms to a bird weighing 4½ lb.). Suffran reported that post-mortem examination showed hæmorrhages, severe congestion in the gastro-intestinal tract, "as well as lesions in many organs," whilst Gallagher found the mucosa of the crop to be white and cooked-looking, with catarrh of the proventriculus; the gizzard mucous membrane stripped easily; the duodenum was congested, and the liver darkened.

Mitchell, Card and Carman (1926) found that chickens might be raised from 9-21 weeks of age on rations containing as much as 8 per cent. salt. For birds of 3-5 lb. weight, the minimum lethal dose was found to be approximately 4 gm. per kilo. body-weight, thus confirming the results of the two earlier workers.

Quigley and Waite (1932) found that recently-hatched chicks endured 30 per cent. salt for short periods, and that it was difficult to put enough salt in an all-

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mash ration to produce an appreciable sudden mortality; while up to 9 weeks of age mortality was not excessive until the mash contained 8 per cent. salt or more. They again confirmed that the minimum lethal dose was 4 gm. per kilo. body-weight.

Halpin, Holmes and Hart (1936), after experiments with chicks from 1-20 weeks old, concluded that 5 per cent. salt in the ration was too much. A 30 per cent. mortality occurred mainly at the 5-8 weeks old period and 2 per cent. salt, although not injurious, was not advantageous, owing to excessive thirst and very moist, watery droppings. A laying trial was carried out with the surviving pullets on the same concentrations, but the 5 per cent. group did not mature so rapidly, laid fewer eggs, and continued to drink more than the controls; there was no mortality in any of the groups.

It was decided to repeat some of this work in order to compare the results and to establish further grounds for diagnosis in suspected cases of salt poisoning sent to the routine post-mortem department.

The work was divided into 3 main parts:—

I.—Experiments with mature fowls.

II.—Experiments with growing chickens, 6-9 weeks old.

III.—Experiments with young chicks.

I. Experiments with Mature Fowls

(a) *Dosing of individual birds.*

Some of the earlier work reported by Suffran, Gallagher and others was repeated in order to observe the symptoms and post-mortem changes in greater detail. Individual mature fowls of both sexes were used, the dose being administered orally by means of a glass funnel and length of rubber tubing, taking the salt, dissolved in water, directly into the crop. Ordinary kitchen salt was used. The birds were all dosed at approximately 4.0 p.m., the results being observed the following morning about 9.30 a.m. after dosing. They were allowed free access to both food and water throughout the experiment.

The description of these experiments is tabulated on pages 160 and 161.

The results of the experiments, in which mature fowls were dosed individually, are in general agreement with those of other workers, except with regard to post-mortem findings. In only one of the 5 birds which died was there evidence of "severe congestion in the gastro-intestinal tract," as described by Suffran (1909), and none showed the "mucosa of the crop white and cooked-looking, with catarrh of the proventriculus," as described by Gallagher (1919).

In all cases where recovery eventually took place, birds were excessively thirsty, and as a result the fæces were extremely moist. Recovery was rapid, being noticeable usually within 24 hours, and there were no after effects. In 3 of the 5 birds to which the dose eventually proved lethal there were marked nervous symptoms, commencing with leg weakness and ending with opisthotony in two cases. In one of the recovered birds there were symptoms of paralysis of one leg, but 24 hours later this bird was normal. Recovery was rapid in all cases provided the bird was able to drink copiously. In each case where the dose was lethal the bird was so ill that it was unable to drink.

There were no definite characteristic post-mortem lesions; the dark, congested appearance of the liver was a constant feature, but no more so than with other

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poisons; the diffuse hæmorrhages on the sub-epithelial layer of the gizzard were striking, but only occurred in two of the five fatal cases; there was a varying degree of enteritis in three cases, but, as mentioned above, it was not very marked.

The lethal dose, as stated by other workers, was in the region of 4 gm. per kilo. body-weight, although death of one mature pullet occurred with a dose of only 3.08 gm. per kilo., and two mature cockerels survived a dose of nearly 4.5 gm. per kilo.

(b) *The feeding of a single lethal dose of sodium chloride in the food to individual birds.*

Four mature Light Sussex cockerels were weighed and the lethal dose in each case estimated (approximately 4.2 gm. per kilo. body-weight). The salt in its normal granular form was mixed into a wet mash and fed to the birds, all food having been withheld for 24 hours. The birds were housed individually in coops. Water was given as required. The results are tabulated in Table I.

TABLE I.

Light Sussex cock. Wt. 5 lb. 2 ozs. Dose 10 g.	Light Sussex cock. Wt. 6 lb. 4 ozs. Dose 12 g.	Light Sussex cock. Wt. 4 lb. 10 ozs. Dose 9 g.	Light Sussex cock. Wt. 5 lb. 4 ozs. Dose 10 g.
	24 hours after receiving salt mash		
Approximately a quarter of the feed left with a high proportion of salt; water pot almost empty; fæces very moist; no other symptoms. Fresh dry mash mixed into the remnants of original feed.	Practically all the feed consumed; water pot empty; fæces very moist; no other symptoms. Fresh mash given.	All the feed consumed; water pot empty; fæces very moist; no other symptoms. Fresh mash given.	Approximately a quarter of the feed and a high proportion salt left; water pot empty; fæces very moist; no other symptoms. Fresh mash mixed into remnants of original feed.
	48 hours after receiving original mash		
All food consumed. Bird normal.	All food consumed. Bird normal.	All food consumed. Bird normal.	All food consumed. Bird normal.

This experiment was repeated about a fortnight later, using the same birds and the same doses, but on this occasion the salt was dissolved in a small quantity of water, the solution being used to moisten the mash. The mixture was again given after a 24-hour fast, but on this occasion all water was withheld for 24 hours after the commencement of the experiment. The results are tabulated in Table II.

From these experiments it would therefore appear very unlikely that fowls living under normal conditions would consume a lethal dose of sodium chloride in a space of time short enough to avoid its partial hydrolysis and elimination, thus preventing any toxic effect. Where the salt in solution was used to moisten the mash, the resulting feed was so unpalatable that only one of the four birds consumed an appreciable amount in the first day, despite the fact that they had been starved for the previous 24 hours.

(c) *The feeding of various concentrations of sodium chloride in mash to adult birds over a long period.*

(i) Ten mature fowls—8 hens and 2 cocks—were housed in pairs in coops. Sodium chloride was added to layers' mash in four different concentrations: 5, 10,

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TOXIC AND LETHAL DOSE

No.	Description	Wt. in lb.	Dose in gms.	Approx. dose per kilo.	Time after dosing
1.	L. Sussex Cock, adult	5.4 lb.	13 g.	5.5 g.	18 hrs. 22 hrs.
2.	L. Sussex Cock, adult	6.8 lb.	15 g.	5 g.	18 hrs. 22 hrs. 24 hrs.
3.	Barred Rock X.L. Sussex Cock, adult	5 lb.	10 g.	4.4 g.	18 hrs. 22 hrs. 48 hrs. 72 hrs.
4.	L. Sussex Cock, adult	8.0 lb.	16 g.	4.4 g.	24 hrs. 48 hrs.
5.	R.I.R. Cock, adult	8.8 lb.	15 g.	3.9 g.	24 hrs. 40 hrs.
6.	L. Sussex Cock, adult	5.8 lb.	10 g.	3.9 g.	18 hrs. 24 hrs. 40 hrs.
7.	L. Sussex Cock, 3 months old	3.6 lb.	5.25 g.	3.3 g.	18 hrs. 24 hrs. 48 hrs. 72 hrs.
8.	W.W. Hen, adult	5.12 lb.	8.6 g.	3.3 g.	18 hrs. 48 hrs. 72 hrs. 96 hrs.
9.	L. Sussex Cock, adult	8.0 lb.	12 g.	3.3 g.	18 hrs. 24 hrs. 48 hrs.
10.	L. Sussex Cock, adult	6.4 lb.	10 g.	3.3 g.	18 hrs. 24 hrs. 48 hrs.
11.	L. Sussex Cock, 3 months old	2.10 lb.	3.75 g.	3.3 g.	18 hrs. 24 hrs. 48 hrs. 72 hrs.
12.	R.I.R. Pullet	5.4 lb.	7.5 g.	3.08 g.	18 hrs. 24 hrs. 40 hrs.
13.	L. Sussex Cock, 3 months old	3.8 lb.	5 g.	3.08 g.	18 hrs. 24 hrs. 48 hrs.
14.	L. Sussex Cock, 3 months old	3.9 lb.	5 g.	3.08 g.	18 hrs.
15.	L. Sussex Cock, 3 months old	3.8 lb.	3.5 g.	2.2 g.	18 hrs. 24 hrs. 48 hrs.

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FOR INDIVIDUAL FOWLS.

Symptoms	Post-mortem findings
Very dejected, squatting on hocks, comb blue and floppy, droppings wet. Dead.	Gizzard mucous membrane (horny epithelium) stripped easily and showed a few diffuse hæmorrhagic areas on underlying tissue—also fairly extensive hæmorrhagic enteritis. Liver congested, a few hæmorrhages on the heart fat.
Bird lying down and unable to stand, comb very blue. Comatose. Dead.	No definite lesions, liver dark and congested, intestinal contents fluid but no enteritis.
Excessive thirst but no other symptoms. Hunched up, very dejected, comb blue. More alert, brighter, eating a little, still very thirsty. Recovered.	
Rather dull, very thirsty, fæces wet. Recovered.	
Very dull and lethargic, complete inappetance and not drinking. Dead.	Crop mucous membrane rather inflamed, a few diffuse hæmorrhagic areas on the sub-epithelial surface of the gizzard, some enteritis, liver and kidneys congested.
Complete inappetance and not drinking, lying down on side. Ditto, spasmodic twitching of legs and repeated swallowing—opisthotony. Dead.	Flesh dark and congested, carcase dry and tacky, heart slightly dilated and coronary vessels engorged, liver and kidneys congested, crop and gizzard normal.
Dull, hunched up and excessively thirsty. Ditto. Still very thirsty. Recovered.	
Very dull, hunched up, not drinking, partial paralysis. Very dull, drinking a little. Eating and drinking. Recovered.	
Rather dejected, excessive thirst, litter wet. Brighter. Recovered.	
Very dejected, comb blue and floppy, drinking. Brighter, drinking, litter wet. Recovered.	
Dull, feathers ruffled, drinking excessively. Ditto and eating. Still very thirsty. Recovered.	
Very dull, hunched up, not eating or drinking, fæces fluid. Crouching down, head back—opisthotony. Dead.	No definite lesions, crop and gizzard normal, liver dark and congested, ovary hæmorrhagic, mesenteric blood vessels engorged.
Dull, excessive thirst, fæces fluid. Brighter. Recovered.	
No effect at all.	
Dull, excessive thirst, fæces fluid. Brighter. Recovered.	

TABLE II.

Light Sussex cock Wt. 5 lb. 2 ozs. Dose 10 g.	Light Sussex cock Wt. 6 lb. 4 ozs. Dose 12 g.	Light Sussex cock Wt. 4 lb. 10 ozs. Dose 9 g.	Light Sussex cock Wt. 5 lb. 4 ozs. Dose 10 g.
	24 hours after receiving salt mash		
Mash hardly touched. Water pot filled.	Approx. half mash eaten; water pot filled.	Mash hardly touched; water pot filled.	Mash hardly touched; water pot filled.
	48 hours after receiving mash		
Very thirsty. Approx. half food eaten; no other symptoms.	Very thirsty; food all eaten; fæces liquid; litter saturated; no other symptoms.	Very thirsty; approx. half food eaten; no other symptoms.	Very thirsty; approx. half food eaten; no other symptoms.
	56 hours		
Normal mash added and the whole readily consumed. Bird normal.	Normal mash given. Bird normal but litter still very wet.	Normal mash given. Bird normal.	Normal mash given. Bird normal.

15, and 20 per cent. The salt was ground into a fine powder and thoroughly mixed into the mash; it was fed, in the first instance, as wet mash, with the addition of a small quantity of water; each pair of birds received a small scratch feed of grain daily and water was given as required. The fifth pair of birds were controls and received ordinary layers' mash.

Feeding of the salt mash was continued for a period of 4 weeks . It was found that the mash was less unpalatable when fed dry, and after 4 days dry mash feeding was adopted. The experimental birds all became excessively thirsty within 24 hours of the commencement of the experiment, and continued to drink large quantities of water over the whole period; their fæces became completely liquid and the litter was saturated, but there were no other symptoms of ill-health. The birds continued to eat the salt mashes in all concentrations, although only in very limited quantities. The control birds remained healthy, although one of them lost weight. See Table III.

At the conclusion of the experiment a post-mortem examination was carried out on each bird. Apart from loss of condition, as indicated by the weight, these birds were all normal, and there was no evidence of any pathological condition.

(ii) The same experiment was repeated, using 8 full-grown R.I.R. cockerels,

TABLE III.

Showing gain or loss of weight over the 4-week period.

Bird	Wt. before exp. lb.	Wt. before exp. ozs.	Wt. after salt feeding lb.	Wt. after salt feeding ozs.	Gain or loss in lb. and ozs. lb.	Gain or loss in lb. and ozs. ozs.	Concentration of salt in mash
Light Sussex hen	5	1	5	4		+ 3	Controls
Light Sussex hen	7	1	6	4		—13	
R.I.R. hen	6	4	6	0		— 4	5 per cent.
White Leghorn hen	4	8	4	2		— 2	
R.I.R. hen	4	6	4	9		+ 3	10 per cent.
R.I.R. hen	6	2	5	13		— 5	
R.I.R. cross cock	6	10	6	13		+ 3	15 per cent.
White Leghorn hen	3	14	4	2		+ 4	
Light Sussex cross cock	5	7	4	4	— 1	3	20 per cent.
R.I.R. hen	6	0	5	7		— 9	

housed in pairs in coops. On this occasion there was no control pair. Concentrations of salt were as before, 5, 10, 15, and 20 per cent. The salt was mixed into layers' mash and fed dry from the commencement; each bird received a small feed of corn daily; water was limited to the normal allowance. The experimental period was again 4 weeks.

As before, each bird became excessively thirsty within a few hours of the commencement of the experiment, and the water pots were emptied each day within an hour or two of being filled; the fæces became completely liquid and the litter saturated; at one stage the birds were noticed to be drinking the fluid collecting on the floor of the coops; the salt mash was obviously extremely unpalatable, and was only eaten because the small corn feed did not satisfy the birds. Two birds died during the experiment (one on the 10 per cent. concentration 20 days after commencement, and the other on the 15 per cent. concentration 23 days after commencement).

Loss of weight during this experiment was more marked than on the previous occasion, and only one of the eight birds maintained its original condition. The loss of weight and details of post-mortem are shown in Table IV.

The results of these two experiments suggest that fowls can tolerate concentrations of sodium chloride up to 20 per cent. in their food, but that it renders the mash so unpalatable, particularly when used wet, that the total intake is insufficient to maintain normal condition; in fact, a state of semi-starvation results.

TABLE IV.

Bird	Wt. before experiment lb.	Wt. before experiment ozs	Wt. after salt feeding lb.	Wt. after salt feeding ozs	Loss in weight lb.	Loss in weight ozs	Post-mortem	Concentration salt in mash
R.I.R. cock	6	8	6	8	—		Both killed in fair condition — visceral organs normal.	5 per cent.
R.I.R. cock	6	6	6	3	—	3		
R.I.R. cock	6	14	6	6	—	8	Killed in fair condition — visceral organs normal.	
R.I.R. cock	4	15	3	10	1	5	Died in an emaciated condition — general atrophy visceral organs; also cystic degeneration left testicle and vas deferens.	10 per cent.
R.I.R. cock	7	4	5	6	1	14	Killed in very poor condition — advanced avitaminosis A.	
R.I.R. cock	6	14	5	5	1	9	Died in very poor condition — carcase very œdematous; heart dilated; liver and kidneys congested; gelatinous exudate over the surface of the liver.	15 per cent.
R.I.R. cock	7	0	6	13	—	3	Killed in fair condition; visceral organs normal.	
R.I.R. cock	6	4	3	15	2	5	Killed in an emaciated condition — visceral organs normal.	20 per cent.

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(d) *The feeding of single meal containing a high concentration of sodium chloride to adult fowls.*

In the following experiments an attempt was made to feed mashes containing a high concentration of salt to groups of birds under conditions identical to those which might occur in the field:—

(i) Nine mature R.I.R. hens housed in a fold unit were given their normal evening meal of wet mash, plus 20 per cent Na Cl. The salt in a saturated solution was used to prepare the mash. The birds had received their normal feed of corn during the morning, and the salted mash was fed in the afternoon; it was readily consumed, not appearing excessively unpalatable, and one hour later the birds were shut into their night quarters, thus preventing all access to their water trough. They were released the following morning, and there was a concerted rush for water, the trough being emptied rapidly; one hen appeared to walk unsteadily, and at midday was found dead; the other eight hens were quite normal, except for the moist nature of their fæces and excessive thirst, which was apparent all day. The normal feed of corn and mash was taken readily.

The dead bird weighed 5 lb. 8 oz.; it was in good condition, but the comb was rather cyanosed. A post-mortem examination showed slight dilatation of the heart with the coronary blood vessels engorged; also a number of small hæmorrhages on the myocardium; the liver was very dark and congested; the crop, proventriculus and gizzard were normal, but there was slight hæmorrhagic enteritis, particularly of the duodenum.

(ii) The above experiment was repeated under the same conditions, but using a fold unit containing 20 R.I.R. cockerels and pullets about 4-5 months old. The concentration of salt in the mash was again 20 per cent. On releasing the birds the following morning, one was found dead and another was lying on its side partially paralysed; the remainder rushed for the water and drank copiously. The paralysed bird showed typical opisthotony 24 hours after eating the salt mash; the head was drawn back and the legs twitched continuously with a paddling motion; it died about 12 hours later.

The first bird which died, a cockerel, weighed 4 lb. 8 oz. Post-mortem examination showed the crop and gizzard to be distended with food; the horny epithelium stripped easily, and there were diffuse hæmorrhagic areas on the sub-epithelial layer; there was also a diffuse enteritis, particularly in the duodenum; the liver was congested, with multiple small hæmorrhages on the surface; the coronary and mesenteric blood vessels were engorged with blood.

The second bird, also a cockerel, weighed 4 lb. 10 oz. Post-mortem examination showed the flesh to be discoloured and congested; the crop and gizzard were packed with food; the sub-epithelial layer of the gizzard again showed diffuse patchy hæmorrhages, and there was slight enteritis; the coronary blood vessels were engorged.

(iii) The experiment was again repeated, using a fold unit containing 14 mature R.I.R. pullets and 4 cockerels. The concentration of salt in the mash on this occasion was 30 per cent. The birds ate the mash, but did not clear it all up, and some of them obviously found it unpalatable. The next morning one pullet was lying on its side partially paralysed; two others appeared very dejected,

but, together with the remainder of the birds, drank copiously as soon as they were let out, and recovered rapidly, although their fæces remained very moist all that day; the paralysed pullet died 24 hours after having consumed the salted mash.

Post-mortem examination showed some evidence of cardiac collapse, but lesions were not marked; the intestinal tract was normal, except for enteritis in the duodenum.

(iv) It was thought that with a lower concentration of salt in the mash than in the previous three experiments, a larger number of fowls might consume a lethal quantity.

The experiment was again repeated, using 7 hens and 2 cockerels housed in a pen, and on this occasion the mash contained 15 per cent. sodium chloride. The mash was fed as before, in the afternoon, and the water containers were removed until the next morning. There were no deaths, and only one of the nine birds showed any symptoms of ill-health, apart from the usual excessive thirst and watery diarrhœa shown by all. The single ailing hen recovered rapidly after drinking copiously.

These experiments proved that a single feet of mash containing a high concentration of salt may cause the death of mature and growing fowls, the mortality presumably depending on the quantity of mash consumed by individual birds. These results are contrary to those obtained where individual birds were fed salt in the mash, but this might be explained by the smaller number of birds used in the former experiment. Even so, the mortality occurring in the three experimental groups is very much less than that frequently reported as having occurred in field cases, e.g., Richardson (1942) reported approximately 17 per cent. mortality, and in various unreported suspected cases encountered recently mortality has been as high as 75-90 per cent.

II. Experiments Using Growing Chickens

(a) *The feeding of mash containing varying concentrations of sodium chloride.*

Thirty-six 2-3 month old R.I.R. cockerels and pullets were placed into battery brooders and divided into four groups. Groups A, B and C contained nine birds each, and the control group, D, consisted of eight birds. Each group was fed for 14 days entirely on National chick mash, to which common kitchen salt had been added in the following concentrations :—

Group A—5 per cent. salt.
Group B—10 per cent. salt.
Group C—15 per cent. salt.
Group D—No salt.

The day after the commencement of the experiment, birds in Groups A, B and C were all excessively thirsty, the water trough being emptied completely within a comparatively short time, e.g., a trough containing four pints was regularly emptied twice in 24 hours by the nine birds, which is equivalent to approximately 18 oz. of water per bird; the control group drank approximately 8½ oz. per bird in 24 hours. Throughout the whole period, Groups A, B and C were very dejected and showed profuse diarrhœa. They did not appear to find the salt mash excessively unpalatable, although Groups B and C (10 per

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cent. and 15 per cent. salt, respectively) consumed considerably less than Groups A or D. Two deaths occurred on the second day, one in Group A and one in Group C—see post-mortem findings below; no other deaths occurred until the thirteenth day, when two birds were found dead in Group C and a third the following day.

The average daily food consumption was:—

Group A—3.05 oz. per bird per day.

Group B—2.2 oz. per bird per day.

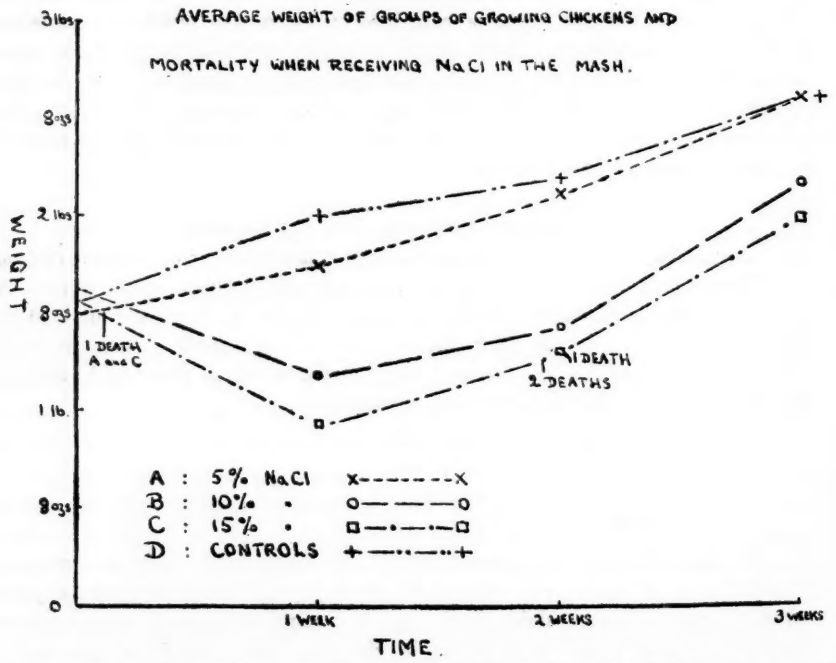
Group C—1.5 oz. per bird per day.

Group D (Control Group)—3.3 oz. per bird per day.

Each bird in the experiment was weighed at the commencement and at the end of each week. At the end of a fortnight all groups were given ordinary chick mash and a few days later put out into a fold unit. They were weighed finally, one week after the end of the experimental feeding period. Water consumption dropped immediately, and the diarrhœa ceased as soon as the salt mash was withdrawn. No further mortality occurred, and the survivors in all groups reared and grew normally.

The average weights of each group and the mortality rate are shown in graph form. (Fig. I.)

FIGURE I.



Post-mortem examination.—The bird ex Group A, which died on the second day of experiment, was in good condition; the whole carcase was rather œdematous; the lungs were congested; there were multiple small petechial hæmorrhages on the myocardium and heart fat; the crop, gizzard and proven-

triculus were normal, but there was a diffuse hæmorrhagic enteritis in the duodenum.

The bird ex Group C, which died the same day, was also in good condition; the hæmorrhages on the heart were more marked; there was again a marked enteritis and the intestinal contents were bloodstained.

The three other birds which died in Group C almost at the end of the experiment were all in extremely poor condition and were obviously in a state of semi-starvation. The visceral organs showed no definite lesions except for a slight enteritis in each case.

This experiment indicates that growing chickens are able to tolerate relatively high concentrations of salt in their diet, and that a sudden severe mortality is unlikely to occur before the general condition of a flock would have aroused the suspicions of the attendants. After about a fortnight, it would appear that the reduced consumption of a mash containing 10 to 20 per cent. salt owing to its unpalatability would result in a flock or group of birds dying gradually from undernourishment. It is of interest to note that two- to three-month-old chickens could apparently tolerate a mash containing 5 per cent. salt without any very marked loss of condition, although throughout the experimental period they were obviously excessively thirsty and suffered continually from diarrhœa; this is in agreement with Halpin, Holmes and Hart (1936).

III. Experiments with Baby Chicks

(a) *The feeding of mash containing varying concentrations of sodium chloride.*

Thirty baby chicks of an average age of about two weeks were divided into three groups and placed in small table brooders. Group I were fed on National chick mash to which 5 per cent. sodium chloride had been added; Group II received the same mash but with 10 per cent. sodium chloride; Group III were the control chicks receiving normal mash. There were two deaths before experimental feeding commenced—one in Group I and one in the control group.

The salt mashes were fed for five weeks, commencing at 4 o'clock one afternoon. The effect was very rapid: at 10 a.m. the next day, both groups receiving the salt mash were showing marked symptoms; the majority were squatting about in a dejected manner; the dropping papers beneath the chicks were sodden owing to a thin watery diarrhœa; the water troughs in each group were completely empty but the chicks were still excessively thirsty; the mashes did not appear to be unpalatable and, after having drunk copiously from the refilled troughs, chicks continued feeding. Three chicks in Group II were showing nervous symptoms; they were crouching down with their heads drawn back and continually twitching. At 4.30 p.m. one chick was dead in Group I; the next day one other was dead in this group and two were dead in Group II, with three more the following day. The symptoms shown by a number of chicks a few hours before death were distinctive. They would stand hunched up, stretching their necks out at intervals and gasping for breath, with their beaks open and eyes shut; they would move with a staggering gait, occasionally giving a spasmodic jump into the air. After 14 days, the two survivors in Group II were added to Group I, all then receiving 5 per cent. salt in the mash.

The death rate in Groups I and II is illustrated in graph form in Fig. II.

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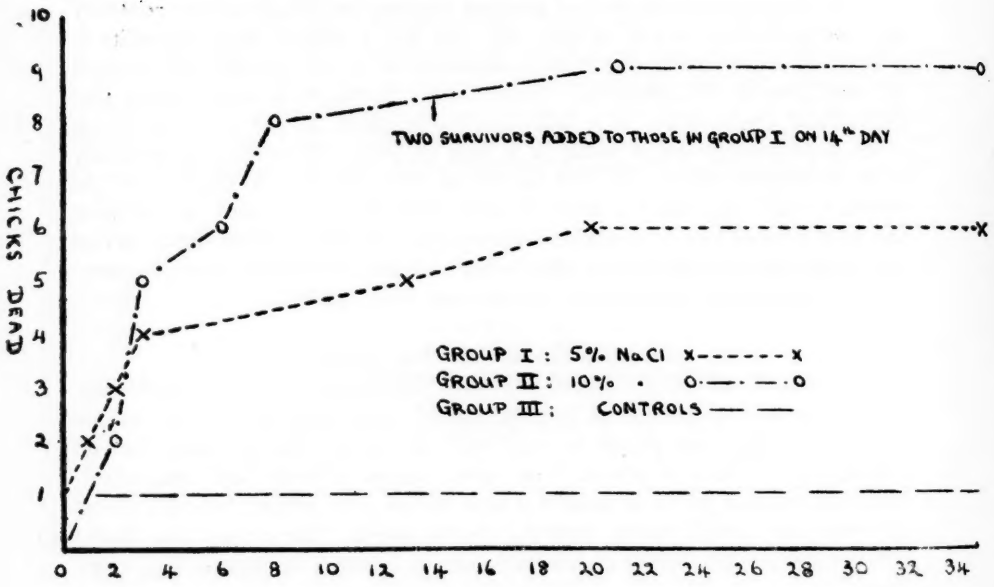
The average consumption of mash over the first five days was approximately:—

Group I—100 grammes per day.
Group II— 45 grammes per day.
Group III—200 grammes per day.

Water consumption was not measured during this experiment.

FIGURE II

MORTALITY RATE IN CHICKS RECEIVING EXCESS OF NaCl IN THE MASH


CHICKS DEAD
DAYS
TWO SURVIVORS ADDED TO THOSE IN GROUP I ON 14th DAY
GROUP I : 5% NaCl x------x
GROUP II : 10% " o—·—·—o
GROUP III ; CONTROLS — —
WEIGHT IN GRAMMES

All the chicks were weighed at the commencement of the experiment and the survivors in Groups I and II, together with the control Group III, were weighed on four other occasions. The average weights of the birds in each group at these times are shown in graph form—see Fig. III.

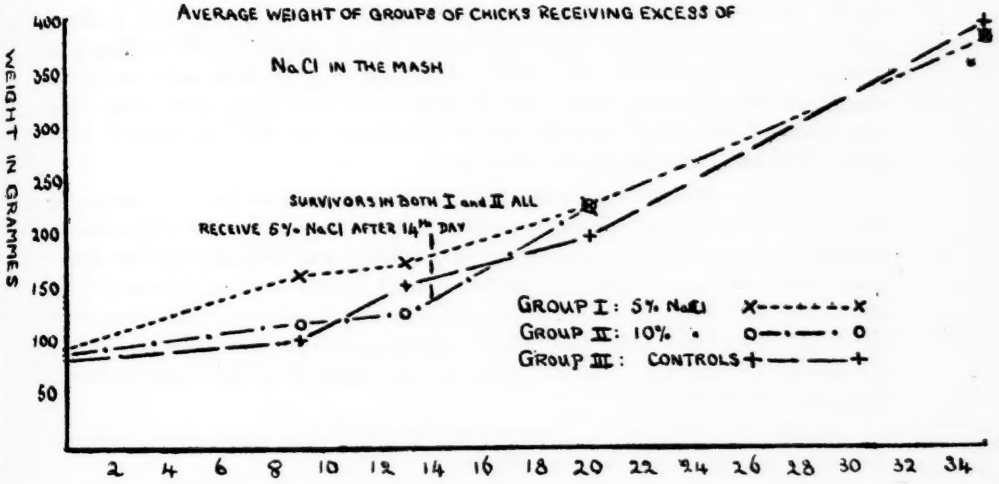
Post-mortem examinations.—All chicks dying were examined post-mortem. The most consistent lesion was a generalised tissue œdema particularly of the subcutaneous tissues and extending in some cases down the legs; the carcases appeared to be waterlogged. In nine cases there was acute congestion of the lungs, the whole organ being a dark purple colour and very œdematous; in these cases also the liver and kidneys were dark red in colour and engorged with blood; the coronary and mesenteric blood vessels were similarly engorged; in three cases there were small hæmorrhages on the surface of the kidneys; the crop and gizzard usually contained a small quantity of moist food, the former frequently being distended with water; there was also a slight enteritis of the duodenum in four cases.

There were no marked differences in the post-mortem appearance of the chicks dying in the first few days as compared with those dying later in the

experiment, except that the general tissue œdema was rather less marked in the latter.

The post-mortem appearance, when considered in conjunction with the symptoms described earlier, suggests that death in chicks suffering from salt poisoning is due to a severe upset of the tissue fluid balance resulting in inability of the kidneys and intestinal tract to remove excess water from the blood stream; in consequence, capillary walls break down all over the body, with flooding of the subcutaneous tissues and congestion of the visceral organs, death eventually occurring owing to cardiac collapse. There was very little evidence that the salt had any inflammatory action on the intestinal tract.

FIGURE III



The five survivors of the experiment were killed and examined post-mortem. All organs and the appearance of the carcases were normal. It is of interest to note that the average weight of these survivors was almost identical with that of the control chicks.

The results of this experiment are in general agreement with those of other workers. It would appear that mortality will occur for the first few days when baby chicks are fed a mash containing about 5 per cent. sodium chloride, but survivors become accustomed to this concentration and growth continues at the normal rate; such chicks, however, continue to drink excessively and suffer from a continual watery diarrhœa.

It is conceivable that a chick mash might through an oversight contain as much as 5 to 10 per cent. sodium chloride, but the sudden onset of the striking symptoms would be so obvious that, under practical conditions, the error would in all probability be discovered before serious mortality had resulted.

(b) *Sodium chloride in the drinking water.*

Thirty baby chicks, one week old, were divided into three groups and placed in table brooders. All three groups received ordinary chick mash, but in Group I the drinking water was replaced by normal saline (0.9 per cent. sodium chloride)

and in Group II by water containing 2 per cent. sodium chloride. Group III were the controls.

Group I.—The next day, several chicks in this group were very dull, the droppings were moist, and they were all excessively thirsty, having already drunk 600 c.c.s of normal saline in the 24 hours. On the second day, at 10 a.m., the water trough was dry, the chicks having drunk another 500 c.c.s; they were feeding and looked rather better than the previous day, but were obviously excessively thirsty; the droppings were more normal. As soon as the water trough was replenished they drank uninterruptedly until their crops were distended with fluid; 700 c.c.s were taken by 4 p.m. on that day; by then several chicks were again dull, and one was gasping with its beak open. At 10 a.m. on the third day another 600 c.c.s had been drunk, making a total of 1,300 c.c.s in 24 hours. Two chicks were dead and the remainder all very dull and huddled round the lamp; droppings again very moist; one or two were still feeding and drinking. By 4 p.m. there was another death and most of them were unable to stand and were showing respiratory distress; some were lying down with their legs stretched out behind. On the fourth day all but one chick in Group I were dead. The survivor died on the fifth day.

Group II.—Twenty-four hours after the commencement of the experiment two chicks were dead in this group and three others very weak, showing evidence of respiratory distress; the remainder were feeding and drinking, but the droppings were very moist; 265 c.c.s had been drunk during the first 24 hours. On the second day at 10 a.m. another 75 c.c.s had been drunk and there were only three survivors; they were very weak, sitting back with their eyes closed and not eating or drinking. On the third day two more died, the single survivor living until the fifth day.

Group III.—The control chicks remained normal, the average water consumption being approximately 300 c.c.s per day for eight chicks.

The death rate in Groups I and II is shown as a graph—Fig. IV.

Post-mortem examinations.—Group I.—The chicks in this group were slightly œdematous and the visceral organs were rather moist, but this feature was not nearly so marked as with the chicks dying from an excess of salt in the mash; the livers and hearts were rather congested; the crops and gizzards were full of food in most cases. The kidneys were all rather pale, slightly enlarged, and showed a slight urate nephritis; the ureters were full of chalky material in each case.

Group II.—The first two chicks to die in this group showed no definite lesions except for darkening and congestion of the livers; the next five showed similar congestion of the livers and also of the hearts; there was no general œdema; the crops and gizzards contained a small quantity of moist food; the remaining three were similar but there was even less evidence of œdema and on the contrary the carcases were rather tacky. The ureters again contained a small quantity of chalky material, but there were no macroscopic kidney lesions.

It will be noted that in two respects the post-mortem findings in this experiment differ from those described in chicks dying from an excess of salt in the food, i.e., the absence of tissue œdema and the occurrence of kidney lesions. Selye (1943) reported similar results when sodium chloride in solution was given to chicks instead of drinking water, but he also described tissue œdema

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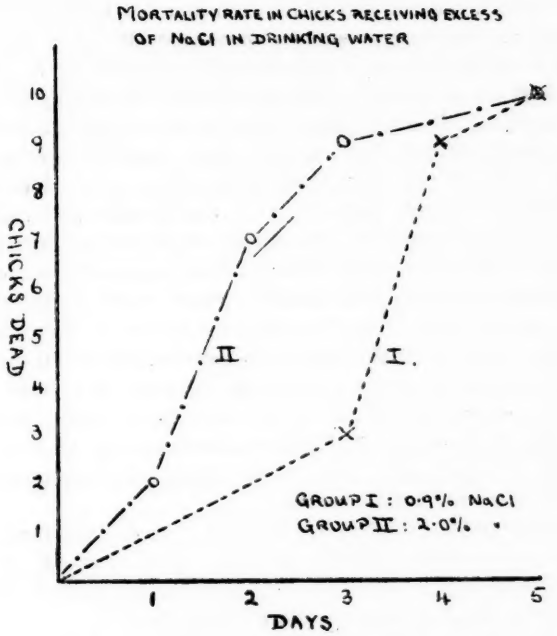
and enteritis as having occurred in the dead chicks in addition to the kidney lesions.

Sodium chloride in solution in low concentrations is definitely more toxic to young chicks than at much higher levels in the food. The practical value

FIGURE IV



of this observation, however, is limited, as it must be a very rare occurrence for any livestock to receive a solution of sodium chloride as drinking water on more than one occasion, as the symptoms produced would be so obvious that the error would be very quickly discovered.

Conclusions

Although approximately 4 grammes of sodium chloride per kilogramme body weight were fatal to mature fowls when placed as a single dose directly into the crop, the same dose mixed into the food was not highly toxic in the case of four mature cockerels housed individually, and no mortality occurred. Mashes containing up to 20 per cent. sodium chloride were fed to 16 mature fowls for a period of four weeks; there was marked loss of condition with the higher concentration and two deaths, both considered to be due to semi-starvation owing to the extreme unpalatability of the mashes. Other symptoms were excessive thirst and a profuse watery diarrhœa. Single feeds of mash containing up to 30 per cent. sodium chloride were given to four groups of mature fowls totalling 56 birds under conditions similar to those existing in most field cases. Only four birds died in all and, apart from excessive thirst and diarrhœa lasting 24 hours after the ingestion of the "salted" mash, the remainder showed no evidence of ill health; recovery was complete and rapid after birds had been

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allowed free access to water. Twenty-seven two-three months old growers were also fed for 14 days on mashes containing up to 15 per cent. sodium chloride; only five birds died (three of the five were on the 15 per cent. mash and died after 13 days in extremely poor condition), and the remainder recovered completely after showing the usual symptoms during the experimental period.

It would therefore appear that, while single feeds of food containing 20 to 30 per cent. sodium chloride may prove fatal to a few individual fowls (probably the most greedy), generally speaking sodium chloride fed over a period to both mature and growing fowls in a high enough concentration to prove fatal is so unpalatable that consumption is reduced, resulting eventually in a low mortality due to malnutrition. It is difficult, in view of these findings, to account for the cases encountered from the field, where sudden mortality, sometimes as high as 75 to 90 per cent. of the fowls, has occurred and is considered to have been caused by sodium chloride poisoning. It would seem that mashes containing a concentration high enough to prove toxic would produce symptoms so marked that the error would be noticed before serious losses had occurred. Recovery on a normal mash and with free access to water would in that case be rapid.

In the case of baby chicks, however, the picture is very different. Where sodium chloride was fed in the mash in a concentration of 10 per cent., a 50 per cent. mortality occurred within three days and after two weeks there had been an 80 per cent. mortality. With a 5 per cent. concentration there was a 30 per cent. mortality in three days. When sodium chloride in solution, even as a normal saline (0.9 per cent.), was given instead of drinking water, a 100 per cent. mortality occurred within five days.

Baby chicks would therefore not tolerate a ration containing a concentration of even 5 per cent. sodium chloride, although, again, symptoms of toxicity would have been so evident that mortality could have been prevented. Sodium chloride in solution in the drinking water is more rapidly fatal.

The post-mortem findings in mature fowls were inconclusive and would not provide evidence on which to base a diagnosis. In chicks, however, the generalised tissue œdema and visceral congestion was most distinctive.

Summary

(1) Sodium chloride was lethal to mature fowls when administered directly into the crop. The lethal dose was approximately 4 grammes per kilogramme body weight, or 30 grains per lb. body weight. Estimated lethal doses when mixed with mash and fed normally failed to produce death.

No mortality could be attributed to sodium chloride poisoning when mashes containing 5, 10, 15 and 20 per cent. respectively of salt were fed to mature fowls over a period of four weeks.

Single feeds of mash containing 15, 20 and 30 per cent. respectively of sodium chloride were fed to groups of adult fowls as far as possible under field conditions, a low mortality occurred with the higher concentrations.

(2) There was no excessive mortality in growing chickens when mashes containing 5, 10 and 15 per cent. respectively of sodium chloride were fed for fourteen days.

(3) (a) Mash containing 5 and 10 per cent. sodium chloride caused heavy mortality within the first week when fed to baby chicks.

(b) There was 100 per cent. mortality in baby chicks within five days when

0.9 and 2 per cent. sodium chloride in solution was given instead of drinking water.

(4) The symptoms and post-mortem findings observed during these experiments are described and the significance of the observations discussed.

The author records his appreciation of the help in the preparation of this paper so kindly given by Mr. R. F. Gordon, Senior Research Officer in the Poultry Diagnosis Department, Veterinary Laboratory, Weybridge.

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Reports

ROYAL VETERINARY COLLEGE AND HOSPITAL

Annual Report for the Academic Year Ended September 30, 1945

We have very much regret in reporting the death of the Most Honourable the Marquess of Crewe, K.G., who was a vice-president of the College for 33 years, from 1911 to 1944.

Teaching

During the session 83 new students were admitted, as compared with 71 in the session 1943-44. The total number of students at the beginning of the session was 317, the number for the previous year being 296.

At the examinations held by the Royal College of Veterinary Surgeons in December, 1944, 106 students presented themselves for examination. and 72, or 68 per cent., passed, and at the examinations held in July, 1945, 256 students presented themselves and 202, or 79 per cent., passed. The percentage of passes in December, 1943, and July, 1944, were 68 per cent. and 79 per cent. respectively.

During the year under report 39 students obtained the Diploma of Membership of the Royal College of Veterinary Surgeons, as compared with 53 who qualified in the previous year.

The number of students preparing for the B.Sc. (Veterinary Science) degree shows a steady increase. Ninety-one were encolled in October, 1944, in all years, compared with 76 in 1943, 68 in 1942, 58 in 1941, and 43 in 1940.

II

Research

(a) In the College

Department of Surgery and Obstetrics.

A study is being made of the effects of ovariectomy on lactation in the cow and of synthetic œstrogens on ovariectomised animals. The investigation into

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certain aspects of bovine sterility, in collaboration with the Department of Pathology, has been continued.

Department of Anatomy.

The work on certain arterial lesions in the horse has been continued.

An investigation is being made into hermaphroditism in the goat.

Department of Physiology.

Studies on circulation have been continued with special reference to circulation through bone in normal and in pathological conditions and to the effects of anoxia on circulation.

The work on respiratory reflexes, with special reference to the reflex control of respiration in the rabbit, has been continued.

Division of Chemistry.

The study of phytotoxins has been continued. A substance of unusual toxicity has been obtained from ricin and attempts are being made to prepare it in a pure crystalline form. The colloidal properties of ricin are being examined in ricin-antiricin mixtures.

Inquiry is being made into the lead content of certain of the viscera of normal animals.

Methods for the concentration of *C. pyogenes* toxin are being investigated in collaboration with the Division of Bacteriology.

Division of Biology.

The work on the relationship between diet and parasitism has been continued.

III

(b) In the Research Institute of Animal Pathology

The pathology of perosis in chicks, including the radiographic study of the development of the hock joint of normal chicks.

Paint poisoning in calves and its experimental reproduction.

Further field trials of the prevention of *Corynebacterium pyogenes* infection in cattle with alum-precipitated toxoid and methods to improve the potency of the immunising agent.

Further study of Johne's disease experimentally induced in goats, and the diagnosis of natural cases of the disease in cattle.

The properties of *Corynebacterium renale* and the course of infection set up by it in experimental animals.

The classification of streptococci recovered from the *cervix uteri* of cattle and from various sites in other domesticated animals.

A study of some causes of pneumonia in calves and pigs.

Investigation of the toxicity of fractions of ricin and the preparation of antisera in goats, in collaboration with the Division of Chemistry.

Further investigation of the life-cycle of *Babesia bovis* in the tick.

The Hospital

At the Beaumont Animals' Hospital attendances at the clinic numbered 14,137, compared with 12,458 during the preceding year. The number of animals seen during the last quarter of the year, 4,566, was the highest for any quarter of any previous year.

Staff—New Appointments

E. O. Longley, B.Sc., M.R.C.V.S., Lecturer, Surgery (Obstetrics).
A. S. King, M.R.C.V.S., Demonstrator, Anatomy.
P. Kiddle, M.R.C.V.S., Demonstrator, Surgery.
D. E. Kidder, B.Sc., A.R.I.C., Demonstrator, Chemistry.
W. Robertson, Demonstrator, Chemistry.
Miss E. Barber, M.R.C.V.S., House Surgeon.

IV

Resignations

A. Messervy, M.R.C.V.S., Lecturer, Surgery.
C. W. Ottoway, F.R.C.V.S., Reader, Anatomy.
J. Bolton, M.R.C.V.S., Lecturer, Surgery.
Mrs. J. G. Wright, M.R.C.V.S., House Surgeon.

Finance

The chief features of financial importance in the year were that part of the Camden Town premises was released from requisition in the last quarter of the year, enabling the teaching of first and second-year students to be resumed in London in October, 1945, and the taking of the West Grinstead Park Estate on a 99-year lease from September 29, 1945. The former explains the decrease in revenue from requisitioned premises and the expenditure under the rehabilitation head. Both will be greater in the following years. The taking on lease of the West Grinstead Park Estate, though it will involve considerable expenditure later, has little financial effect for the present.

General College expenditure exceeded income, excluding Government grant, by £26,004, as compared with £22,407 in the preceding year. Revenue expenditure amounted to £46,346, compared with £42,416 in the previous year. The increase was due partly to rising establishment charges and partly to expenditure on rehabilitation. Income, excluding Government grant, showed little change. There was an increase of £1,028 in the income from students' fees, but rent of requisitioned premises fell by a like amount. Subscriptions were received from 347 regular subscribers.

The net cost of the Beaumont Animals' Hospital at Camden Town was £1,298, compared with £1,457 in the previous year.

BRUCELLOSIS OF SWINE*

[Report of special committee, Illinois State Veterinary Medical Association, Springfield, January 17-18, 1946. Dr. A. K. Kuttler, Springfield, chairman; Drs. C. R. Collins, Dixon; Dr. Lloyd Darst, Princeton, and Dr. W. W. Warnock, Aledo.]

Interest in brucellosis of swine has increased among Illinois breeders during 1945. The agglutination test is of greater importance in diagnosing brucellosis in swine than in cattle, due to the fact that there appears to be a larger percentage of swine which will abort as a result of brucella infection, without being observed by the owner, than is the case with cattle. Therefore, the agglutination test should be adopted for all breeding herds. The entire breeding herd should be tested, and if reactors are found in dilutions which should be

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run 1 :25 or lower, the entire herd should be considered infected and disposed of for slaughter; starting with a new herd known to be free from infection.

In valuable breeding herds in which this practice is considered economically unsound the entire herd should be tested, and pigs should be removed from infected to clean premises at weaning time. Test gilts and boars up to and including the first pregnancy. Breed only those gilts negative to 1 :25 dilution to non-infected boars.

Owners should be warned of the greater danger of developing undulant fever through exposure to brucellosis in swine, also, that the swine type may be transmitted to cattle. There is no evidence that the bovine type is a serious factor so far as spreading from cattle to swine is concerned. No satisfactory means of immunising swine has been developed. Research along this line should be continued.

The committee believes if this procedure is carefully observed it will result in real dividends to swine producers. Your committee recommends more educational work and publicity be given to brucellosis in swine. Your committee further recommends that regulations be adopted by the Illinois Division of Livestock Industry for the control of brucellosis in swine, and that the secretary of the Illinois State Veterinary Medical Association be instructed to furnish a copy of this report to Dr. C. E. Fidler, chief veterinarian; a copy to Dr. C. C. Hastings, Williamsville, Ill., practicing veterinarian, appointed by Governor Green as a member of the Advisory Board of Livestock Commissioners; and C. W. Mitchell, Springfield, Ill., appointed by Governor Green as a representative of the swine producers on the Advisory Board of Livestock Commissioners.

[* Received by the courtesy of Dr. A. H. Quin, Head of the Department of Clinical Veterinary Medicine, Jensen-Salsbery Laboratories, Kansas City.

SUPPLEMENTARY VITAMINS FOR CALVES*

[DIGEST of paper presented by William E. Krauss, Chief, Department of Dairy Industry, Ohio Agricultural Experiment Station, before the sixty-fourth annual meeting of the Illinois State Veterinary Medical Association, Springfield, January 17-18, 1946.]

BEFORE discussing the need for supplementary vitamins for calves their vitamin requirements must be considered, and the extent to which natural feeds can supply those needs. Growing calves' vitamin needs will be satisfied by meeting the following recommended allowance per animal: Weight, 50 lb., 150 I.U. vitamin D; 100 lb., carotene 6 mg., 10,000 I.U.; vitamin D, 300 I.U.; with virtually proportionate increases for 150, 200 and 400 lb. (Thiamine, riboflavin, niacin, pyridoxine, pantothenic acid and vitamin K are synthesised by bacteria in the rumen, and it appears that adequate amounts are furnished by a combination of rumen synthesis and natural feedstuffs.) On the above basis, it would seem that vitamins A and D are the only ones needing attention. Can natural feeds supply adequate amounts?

The vitamin A and D content of milk varies according to the feed of the cows, and, in the case of vitamin D, also according to the degree of exposure of the cows to sunlight. Average summer milk can be expected to contain about

[* Received by the courtesy of Dr. A. H. Quin, Head of Department of Clinical Veterinary Medicine, Jensen-Salsbery Laboratories, Kansas City.

1,800 I.U. of vitamin A per quart, and average winter milk about 1,100 I.U. The vitamin A content of colostrum can be said to contain from several to ten times as much vitamin A activity as normal milk. Vitamin D content of milk may range from 6 or 8 units per quart to a maximum of 50. Colostrum has been reported to contain several times as much vitamin D as does normal milk.

If it is assumed that a new-born calf weighs 75 lb. and consumes an average of 6 lb. of colostrum daily for three days, daily intake of vitamin A would be at least 10,000 units. Its daily vitamin D intake would be about 100 units. Vitamin D intake thus would be a little short. If it is assumed further that during the following month the calf receives an average of 10 lb. of pooled herd milk daily the intake of vitamin A would be about 7,500 units, and of D about 200. Vitamin A might be a little short, but it must be remembered that the requirement is expressed in terms of carotene, whereas a considerable amount of the vitamin A potency of milk is supplied by true vitamin A, more efficiently utilised than carotene. Again the vitamin D intake would be short.

If, in addition to milk, a calf of this age consumes an average of ¼ lb. grain and ¼ lb. hay the requirements for both vitamins A and D would be fully met, especially if a fortified calf-starter were used. (The range of possibilities, depending on the nature of the feeds, is indicated in the table.)

	Carotene. I.U.	Vitamin D. I.U.
Allowance for new-born calf, 75 lb.	6,500	225
Supplied by 6 lb. low-potency colostrum ...	13,500	120
Supplied by 6 lb. high-potency colostrum ...	45,000	360
Allowance for 100 lb. calf	10,000	300
Supplied by 10 lb. winter milk	5,500	40
Supplied by 10 lb. summer milk	9,000	250
Supplied by ¼ lb. grain...	275	—
Supplied by ¼ lb. fortified calf-starter ...	900-1,800	125-250
Supplied by ¼ lb. sun-cured hay	4,000	50-150

At this point whole milk is discontinued and a shift made to skim-milk, or complete dependence is placed on a calf-starter, hay and water. Vitamins A and D from then on must come from the grain or the calf-starter and from the hay.

For a 200 lb. calf requiring 20,000 I.U. of carotene and 600 of vitamin D, 2 lb. of grain would supply 2,200 I.U. of carotene and no vitamin D. Two pounds of calf-starter would supply 7,200-14,000 of carotene and 960-2,000 of vitamin D, and 2 lb. of sun-cured hay 32,000 carotene and 400-1,200 vitamin D. Figures for a 300 lb. calf would be uniformly 50 per cent. higher throughout.

It thus would seem that until the calf is consuming at least 1 lb. of sun-cured hay and 1 lb. of fortified calf-starter some need exists for supplementary feeding of vitamins A and D, especially in the winter milk feeding period. Some additional benefit, reduction in incidence of scours and pneumonia, may be obtained from supplying two or three times as much vitamin A as is indicated in the table of allowances. There also is evidence pointing to the use of true vitamin A rather than carotene for young calves, and that the need for vitamin A is greater in the winter than in the summer. The use of a vitamin D supplement is almost imperative, as is shown by the fact that even with good roughage available, rickets can occur because of over-feeding on milk and unfortified grain.

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In a calf capable of consuming 1 lb. of grain and 1 lb. of hay daily, the rumen should be developed to the point of at least part synthesis of thiamine, niacin, pyridoxine, riboflavin, pantothenic acid and vitamin K, and ascorbic acid synthesis throughout the body also should be operative. Supplementary feeding of these in the case of calves would then need to be confined to the milk feeding period, if need for such supplementation could be shown. The calf's requirements for the factors of the B-complex, vitamin K and ascorbic acid are not known, and their value as milk supplements can be determined only by trial and error.

From work at the University of Wisconsin has evolved the interpretation that the use of vitamin A, niacin and ascorbic acid from birth through 3 weeks of age would reduce the incidence of scours and navel ill in calves. This has led to the rather promiscuous sale and use of tablets and capsules containing vitamin A, niacin and vitamin D, with or without ascorbic acid.

Interest aroused by the Wisconsin work made it seem desirable to attempt a large-scale experiment-demonstration that might help evaluate the use of such vitamin supplementation under farm conditions. Consequently, in co-operation with the Department of Agriculture of Michigan and the Department of Public Welfare of Ohio, arrangements were made to feed gelatin capsules to every calf born in some of the herds of these departments, as well as in the herds of Michigan State College, the Ohio Experiment Station and the Ohio State University. Two sets of identical gelatin capsules were prepared by the Gelatin Products Corporation, Detroit. Each capsule of one set, coloured red, contained 10,000 I.U. vitamin A, 300 I.U. vitamin D, 250 mg. ascorbic acid, and 50 mg. niacin. The other set, coloured blue, contained an equal weight of biologically inactive oil.

Each herdsman was given a supply and record blanks, and instructed to feed either a red or a blue capsule on the day of birth and for 19 days thereafter. The difference between the capsules was not told. Initial weights and periodic weights thereafter were obtained where scales were available; heart girth measurements in the other instances. Nine herds were used in Michigan, 8 in Ohio. Blood data were obtained on the calves in the college and experiment station herds in Ohio and in the college herd and some of the state herds in Michigan. Feeding and management practices of the various institutions were followed for all calves. The experiment continued through one year, to include all seasons.

A modification of the general procedure in the herd at Ohio State, where red and blue capsules were superimposed on groups of calves receiving colostrum for 3 or 7 days, demonstrated the value of colostrum as a source of vitamin A and the advantage of extending the colostrum feeding period.

From this large-scale study it was apparent that the daily administration to calves, during the first three weeks, of vitamin A, vitamin D, ascorbic acid and niacin did not accelerate growth, did not prevent the occurrence of scours, and did not eliminate mortality from scours and other causes. Further, as measured by the same criteria, the performance resulting from such vitamin supplementation was not significantly different from that of suitable controls. The only significant finding was that the vitamin A level of the blood of calves fed the vitamin supplement was somewhat elevated above that of the controls.

The results of this experiment are in agreement with those obtained at

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Cornell on fewer calves, but under careful control. They differ from the Wisconsin result, but according to some of the Wisconsin published work the calves did not receive colostrum and were maintained on skim-milk.

The key to the problem would seem to rest in the nutritive value of the colostrum and milk fed to the young calf. This, in turn, is a reflection, so far as vitamins A and D are concerned, of the quality of the roughages fed to the dams and to the rest of the milking herd. It is conceivable that when the roughage is poor the nutritive value of the milk may be low enough not to meet the requirements of the calf, in which case vitamin supplementation might prove effective. It is also conceivable that when nutritional scours does occur even higher dosages than are used in the one-a-day method may be given with beneficial effect.

Until widespread nutritional deficiency among calves can be demonstrated, until the B-complex requirements of calves are known, and until knowledge regarding the nutritive value of colostrum is more complete, general use of vitamins for calves cannot wisely be recommended. Certainly the use of vitamins cannot be expected to overcome the ill-effects of poor management practices.

ILLINOIS MASTITIS CONTROL PROJECT*

[Summary of address by Robert Graham, D.V.M., Dean, University of Illinois College of Veterinary Medicine, sixty-fourth annual meeting of the Illinois State Veterinary Medical Association, Springfield, January 17-18, 1946.]

The present programme of bovine mastitis control in Illinois supported by federal funds will terminate June 30, 1946. This programme, in effect in 38 counties since April, 1944, was sponsored as a war emergency measure by the Extension Service of the Illinois State College of Agriculture to increase milk production. In addition to the increased milk produced during the emergency, the "know-how" in the control of bovine mastitis has been perfected and extended by veterinary practitioners in co-operation with Illinois dairymen. The results of this war effort now can be incorporated into a post-war programme, through a co-operative arrangement between dairymen and veterinarians, working with the Extension Service of the State College of Agriculture.

Already more than 50 veterinary practitioners in different localities of Illinois are working with their dairymen on problems of diagnosis and treatment of infected animals. The Extension Service of the College of Agriculture urges all practicing veterinarians to familiarise themselves with the Illinois plan, and to go forward with the dairy industry in their fight against the disease.

Under the emergency programme, 7 veterinarians, known as war food assistants, were located in the principal milk producing area of Illinois to work with practising veterinarians and dairymen. These men worked in more than 600 herds and conducted approximately 50,000 tests in detecting infected animals. During the past year more than 100 barn meetings were sponsored by farm advisers co-operating with the Extension Service in order to demonstrate effective preventive and control measures. These meetings were attended by more than 4,800 farmers. The incidence of bovine mastitis in 674 co-operating herds in the project was reduced on an average of approximately 17 per cent., while a total of 80 infected herds was cleaned up in a period of 18 months. Only a few herds

* Received by the courtesy of Dr. A. H. Quin, Head of Department of Clinical Veterinary Medicine, Jensen-Salsbery Laboratories, Kansas City.

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enrolled were found clean on the initial test. This significant observation clearly shows that certain Illinois dairymen already had mastered control of this disease by good methods of management and prompt attention to affected animals. The continued success of this programme is dependent upon the widespread adoption of simple and practical management methods to suppress and control this disease.

Ninety-five per cent. of the job is the dairyman's every day responsibility. The local veterinarian may demonstrate proved procedures of good management, and also give wise counsel in checking the spread of the disease by early diagnosis and prompt treatment of infected animals.

Practising veterinarians may submit milk samples for diagnosis. The University laboratory will continue to make these examinations without cost. Further assistance in mastitis prevention and control may be obtained from milk processing plants and other organisations of the dairy industry. Co-operation between dairymen and veterinarians is the best known approach in preventing and controlling bovine mastitis. This relationship with the assistance of the Extension Service of the College of Agriculture is available to all dairymen and veterinarians in Illinois.

Abstracts of Current Literature

THE OCCURRENCE IN BULL SPERM OF CERTAIN "MEDUSA FORMATIONS"*

On morphological examination of sperm samples from 200 bulls being regularly bred, a so-called "Medusa formation" was observed in 10 cases. These previously undescribed bodies were approximately 2-5 μ in diameter, i.e., the size of, or a little larger than, the head of a normal sperm cell, and consisted of a deeply staining base with 10-30 filiform projections about 10 μ in length (c.f., tail of normal bull sperm, which measures 60-70 μ). The bodies were subsequently shown to be detached fragments of the ciliated coat covering the epithelial cells which line the efferent tubules of the testis. The demonstration of these bodies in sperm samples is taken by the present author to lend support to the theory advanced by Hammar (1897), who postulated that there was alternation between a ciliated phase and a secretory phase in the epithelial cells of the efferent tubules of the testis.

The author points out that the "Medusa formation" take most ordinary biological stains poorly, and that but for the methyl violet stain employed in this investigation, which overstains all cell elements intensely, these fragments of the ciliated coat would not have been noticed. By detailed and thorough examination, it seems possible that "Medusa formations" would be demonstrable in any sample of bull semen. A limited investigation carried out with great care on three ejaculates from three bulls, which at a previous less careful examination had not shown "Medusa formations," revealed their presence in two samples after considerable perseverance. In an addendum, the author states that he had been able to demonstrate the presence of "Medusa formations" in semen samples from five stallions. N.J.S.

* Blom, E. (1944): "On the Occurrence in Bull Sperm of Certain 'Medusa Formations' Derived from the Epithelium of the Efferent Ducts of the Testis." *Acta. Path. Microbiol. Scand., 21,* 713-720.

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SPONTANEOUS DETACHMENT OF THE GALEA CAPITIS IN SPERMIA OF THE BULL AND STALLION*

THE head cap, or *galea capitis,* of the mammalian spermatozoon consists of a thin cytoplasmic structure covering the anterior part of head of the spermatozoon. Its function is not known, and there is a lack of agreement among different authorities as to whether it occurs in all mammalian species or not. Blom, in the present article, confirms the presence of a cap in the spermatozoon of the bull and of the stallion, previously described by Ballowitz (1891) and by Retzuis (1909).

The author, using Indian ink preparations of sperm after the method described by Burri for bacteriological preparations or in sperm smears stained with alkaline methyl violet, has succeeded in demonstrating in bull sperm the occurrence of a spontaneous, complete detachment of the cap from the head itself. The detached caps appear as characteristic, thin structures resembling "bathing caps" lying free among the spermatozoa.

Sperm samples from bulls that were being regularly bred usually contained either no detached *galea* or less than 1 per cent. of the sperm by differential count. On the other hand, a high percentage of detached caps was invariably found in the sperm of bulls which had not been bred for a long period (three weeks or more). In two bulls whose semen was followed over some length of time, it was found that in the first ejaculate taken after a prolonged sexual rest detachment of the cap was present in 16 per cent. and 7 per cent. of the spermatozoa respectively. When semen collections were made at short intervals no detachment of the *galea* could be demonstrated. A similar finding, where the percentage of detached caps varied between 8 and 18 per cent., was made in the case of a four-year-old bull belonging to an artificial insemination centre, and which, on account of slowness to ejaculate into an A.V., was unsuitable for regular, routine semen collections. Sperm from this bull, taken at long intervals, was of normal density, but of poor motility and low fertilising capacity. When, in the course of a trial requiring great patience, seven collections were made in one week, the sperm showed relatively good motility and had a low percentage (3.2) of detached caps. This bull was subsequently sold for natural service on a farm, where he showed normal libido and fertility.

These findings would appear to indicate that a high percentage of cap detachment in a sperm sample arises from a long period of non-breeding, and is attributable to the regressive changes which spermatozoa undergo in the *canda epididymis.* Detachment of the *galea* is one of the first degenerative changes to become observable.

The author suggests that the proportion of spermatozoa showing detachment of the *galea capitis* in a semen sample may have some significance in evaluating the fertility of bulls, particularly when the number of detached *galea* fails to decreased markedly in several ejaculations taken at short intervals. In this latter eventuality the author thinks that such a finding "indicates a certain degree of sexual sluggishness in the bull that possibly may make him unsuitable for use in artificial insemination." N.J.S.

* Blom, E. (1945): "Spontaneous Detachment of the *Galea Capitis* in Spermia of the Bull and Stallion." *Skand. Vet.-Tidskr., 35,* 779-789.

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Reviews

The Artificial Insemination of Farm Animals. Edited by ENOS J. PERRY, New Brunswick. Rutgers University Press, 1945; pp. 265. Price $3.50.

ARTIFICIAL insemination has been designated as the most striking contribution which science has made to animal husbandry in recent years, and this method of breeding has become the subject of the greatest interest to all sections of the livestock production industry. Although originally developed in Russia during the early part of the present century by E. I. Ivanoff, it was not until about 1936 that artificial insemination created much interest elsewhere. From this time onwards, however, the potentialities of artificial breeding came to be realised in many countries, and to-day the method is firmly established as a practical procedure for the breeding of all kinds of livestock, but notably for dairy cattle. In the last decade, too, considerable advances have been made in the knowledge of reproductive physiology and in its application to artificial breeding. This has inevitably resulted in the accumulation of a voluminous literature, both scientific and popular, concerning all aspects of artificial breeding, much of which is easily available only to the specialist worker, and most of which would prove hard, if not altogether unintelligible, reading for the average farmer or livestock breeder. This book, produced under the editorial supervision of Professor Perry, of Rutgers University, provides a popular but authoritative exposition of the principles, practice and objectives of artificial insemination as applied to farm livestock; it should, therefore, have considerable appeal and usefulness to everyone interested in the problems of animal production, but especially to the smaller and progressive breeder of animals for whom it is primarily written.

The book opens with a short historical introduction to the development of organised artificial breeding, and discusses the advantages and limitations of the method. There then follows an elementary account of the male and female reproductive organs and a general description of the main operations involved in the practice of artificial insemination. This latter subject is developed in successive chapters, which separately deal with the procedures of semen collection and insemination as applied to cattle, sheep, horses and jackstock, poultry and pigs. In these chapters the advantages and disadvantages of the differing techniques and methods for each species are carefully described and explained.

Three chapters are devoted to the application of genetics to livestock improvement, and include such topics as systems of breeding and methods for the selection of sires.

For those connected with the running of artificial insemination centres in Great Britain, one of the most interesting chapters of the book will be that detailing the organisation of co-operative breeding associations in the U.S.A. It will be seen that the problems connected with large-scale artificial breeding are almost identical in both countries, e.g., finding good proven bulls that are fertile, convincing new members that not all cows conceive with one or two inseminations, overcoming the difficulty when artificial breeding has been oversold, etc.

The book concludes with two chapters, "Disease in Relation to Artificial Insemination" and "Feeding and Management of Sires," which are the least satisfactory of the whole book. The importance of genital disease, and more

particularly the methods by which it may be disseminated, to the efficiency of reproduction, is such that the editor might with advantage seek the collaboration of a veterinarian experienced in the disorders of reproduction when a future edition of the book is called for. The chapter dealing with the feeding and management of sires is only an outline, and is written in too general terms to be of much practical value.

There are a few mispellings of technical terms, e.g., in Chapter III, "epididymus" for "epididymis," "œstrous" when "œstrus" is meant, but the text is singularly free from typographical errors. Most of the chapters are illustrated by excellent diagrams and figures. There are several statements which are presented as facts, but which are either erroneous or are stated too categorically to convey their correct implication; this, however, probably cannot be avoided in a book written for the layman. Among these may be cited "telegony . . . held the undivided attention of breeders" (p. 4); "it was proved impossible to cross . . . the dog and the fox, and the European rabbit and hare" (p. 7); "individual males *undoubtedly* inherit varying rates of testicular activity (p. 15); "the low surviving capacity" of horse sperm in the female reproductive tract (p. 101); "the sperm cells . . . utilise as a source of energy the sugars present in the semen" (p. 113); c.f., "after leaving the male it (the sperm) is incapable of using external sources" (of energy) (p. 20). A surprising omission from the section dealing with evaluation of sperm is that no mention is made of "wave motion," a feature to which in this country, at least, considerable importance is attached.

Despite these criticisms, the book is a useful addition to the literature dealing with animal reproduction, and many veterinarians should find it of value for their own information and also for that of their clients who require enlightenment on the methods and principles of artificial breeding. The book will, without doubt, also serve as a useful text in the training of lay inseminators.

N.J.S.

Principles and Methods of Animal Breeding. By R. B. KELLEY, D.V.Sc., Animal Geneticist, Council for Scientific and Industrial Research, Australia. Sydney: Angus and Robertson, Ltd., 1946; pp. 296. Price 15s.

This book is the outcome of a series of refresher courses which the New South Wales branch of the Australian Veterinary Association, in conjunction with the Sydney University Veterinary Faculty, is conducting for its graduate members and for officers of the Agricultural Department. The subject for one such course, lasting two weeks, is to be Animal Breeding, and Dr. Kelley now presents in book form the series of lectures which he will deliver on this subject. His aim has been to discuss the principles and methods of animal breeding, and to associate established practices with biological precepts. It can be said that Dr. Kelley wholly succeeds in his objective; and it is seldom that a reviewer is afforded the opportunity of recording such unqualified approval as it is possible to do in the case of this book.

The book commences with a well-documented survey of the formative period (from about 1735 to 1860) of British livestock breeding. The methods by which the great "improvers" of the past produced the Shorthorn, Hereford and Aber-

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deen Angus breeds of cattle are clearly and concisely explained; reference is also made to the evolution of the Thoroughbred and Clydesdale horses. The important point emphasised is that almost without exception the "improved" breeds of livestock had their origin in a heterogeneous mixture of relatively unimproved types, and that this mixed state of the early populations was fundamental to the procedure of selection. Once the "improvers" had obtained animals approximating to their ideal type, they were then deliberately isolated for breeding purposes into larger or smaller groups. When the isolation group was relatively small (herds or families), the mating of closely related individuals was inevitable, and so was established the practice of in- and in-breeding, which was so characteristic of the early construction period of most of our breeds of livestock.

This historical review of breed formation provides a background for a discussion of the more abstract aspects of animal breeding, in which the author presents the modern concept of genetics and its application to livestock improvement in a manner which holds the reader's interest in every detail. But Dr. Kelley is never content with abstract theories alone, but offers proof of his statements by reference to actual pedigrees and concrete examples in animal breeding. The result, in our opinion, is the best short exposition of applied genetics so far achieved in the literature of this subject. The section dealing with the relations between environment and heredity is particularly convincing by reason of the data which the author marshals to emphasise the need for a realistic interpretation of the opposing forces operative in any system of selection.

The book concludes with a consideration of artificial insemination, progeny-testing of fine-wool sheep, and current research, all of which are treated with special reference to Australian conditions. An explanation of Wright's method of estimating the degree of in-breeding and relationship is given in an appendix. There is a selected list of references, which should serve as a guide to anyone who wishes to explore a wider field of literature.

The great value of this book lies not only in the great amount of information so discriminately wielded together, but also in its clear exposition and balanced arrangement. The writing is always lucid and the examples apt; wearisome academic discussion of genetical principles is as far as possible omitted, yet there is no sacrifice of accuracy. This is a book which should be read by all veterinarians; whether graduate or undergraduate, they cannot fail to profit greatly from a study of its pages. Finally, it is fitting that we should congratulate our Australian veterinary colleague on the excellence of his book, which, we venture to say, will be for many years to come a standard work of reference on the principles and methods of animal breeding. N.J.S.

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